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CLINICAL AND EXPERIMENTAL

A STUDY OF THE GRAM-POSITIVE COCCI PRESENT IN THE VAGINA AND CERVICAL CANAL OF THE UTERUS*

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THE gynecologists recognize the Gram-positive cocci as important constituents of the bacterial flora of the vagina and the cervical canal.[1] These cocci are almost always present in cultures obtained from this part of the female genital tract. We were interested in the question of the relation between various cocci present in vaginal and cervical regions and also between those of the vaginal and fecal cocci. We have used certain cultural and biologic characteristics to differentiate the groups, such as sugar fermentations, limiting hydrogen-ion concentration and pathogenicity for mice.

TECHNIC

Cultural media were the same as those already described.[2] The methods for the determination of the limiting hydrogen-ion concentration[3] and pathogenicity for mice[4] were the same as we have previously employed. The swabs containing the material for bacteriologic examination were obtained in the following manner: After a speculum was inserted into the vagina, swabs were obtained from the sides of the vagina between the blades of the speculum at the respective levels. The cervix was cleaned with sterile absorbent cotton by repeated application, then a swab was inserted in the cervical canal. All swabs were put in dry sterile test tubes and used to seed the respective media within three hours after being obtained. Our material was taken from patients in the out-door dispensary of Mercy Hospital; all had a leucorrheal discharge and pathologic lesions associated with such discharge.

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OBSERVATIONS

The appearance of colonies of staphylococcus was noted. These were typical colonies; only a few hemolytic varieties were encountered. The blood agar plates were examined by transmitted light and distinct greenish areas were observed around the (streptococcus) colonies. Some colonies appearing as the "indifferent" variety would upon subculture produce some methemoglobin. Subcultured into vitamine bouillon,[2] the staphylococcus always produced a uniform cloudiness, and sedimentation became apparent after incubations of eighteen to twenty-four hours. The streptococcus in this medium varied from a uniform cloudiness to a typical granular sediment adhering to the sides and settling to the bottom of the tube; the bouillon remained perfectly clear. All gradations between these two extremes were observed with Gram-positive cocci that grew in chains. Morphologic studies were always made upon smears taken from plain vitamine bouillon (P_H 7.6-7.8) after eighteen to twenty-four hours' incubation.

We found many colonies on blood agar that were small (1 mm. or less) granular, lusterless, firm, grayish, (streptococcus) with a dark brownish color noticeable around the colony on transmitted light. These colonies were smaller than the streptococcus colonies and the color was not observed unless plates were carefully studied against a strong light. When colonies of this type were subcultured to bouillon, the morphology of a smear from a twenty-four-hour-old colony was that of single and diplococcus. Chains were never formed; at times four members in diplo-arrangement would be adherent, or the same number in tetrad-arrangement. Subcultured for several days upon bouillon, with intermittent blood agar plate transfers, the morphology of these cocci was not changed. The growth in the bouillon (P_H 7.6-7.8) was nearer the streptococcus than the staphylococcus type of growth. The sediment was seldom granular, and usually showed slight viscid, stringy shreds when agitated; sedimentation was mostly collected on the sides of the tube and only settled to the bottom after agitation. This type of coccus has probably been considered as a streptococcus by other workers. We have found the morphology on smears from a twenty-four-hour-old bouillon culture to be different from both the streptococcus and the staphylococcus. Certain cultural characteristics help to differentiate it from the streptococcus, but it resembles the streptococcus much more than it does the staphylococcus. This coccus resembles the enterococcus described by Thiercelin[5] and recent investigations by Dible[6] seem to differentiate an enterococcus from the streptococcus fecalis group of Andrews and Horder.[7]

We have not investigated this coccus sufficiently to warrant our calling it an enterococcus in the sense implied by Weissenbach[8] and other French investigators. But we feel that if the cocci of this group were classed as streptococci, our incidence of streptococci would be too high and we have found the characteristic previously mentioned to be constant for this group of cocci. We will refer to this cocci as the enterococcus in this paper, meaning by that term a Gram-positive coccus; the morphology on smears from a twenty-four-hour bouillon culture shows a single and diplococcus, with isolated cocci in

chains of four members or in groups of four members. On blood agar plates the colony is small (1 mm. or less), gray, granular, lusterless, with a brownish discoloration of the environment when examined by transmitted light.

We have used lactose, mannite and salicin as fermentable substances in previous work with streptococci. In this work we have used the same substances for purposes of comparison. The number of fermentable substances must for technical reasons be limited in most bacteriologic work in connection with clinical material.

EXPERIMENTS

One hundred seventy strains of Gram-positive cocci were isolated from the vaginal tract, forty-eight staphylococcus, sixty-six enterococcus and fifty-six streptococcus. Two hundred sixty-two strains were isolated from the cervical canal of the uterus, seventy staphylococcus, ninety-six enterococcus and ninety-six streptococcus. Table I shows the reactions of these strains according to their ability to utilize lactose, mannite and salicin as carbon sources of energy.

Mannite fermentation has been found to be rather constant for most fecal strains of cocci, especially the streptococci.[7] In Table II, the mannite fermentations of vaginal and cervical strains of streptococcus are compared with those isolated by Oppenheim from the feces and by Arnold from the pharynx. It will be noticed that the vaginal strains are nearer the fecal strains in their

TABLE I

	VAGINAL TRACT				CERVICAL CANAL			
			STREPTOCOCCUS				STREPTOCOCCUS	
	STAPHYLOCOCCUS	ENTEROCOCCUS	NON-HEMOL.	HEMOL.	STAPHYLOCOCCUS	ENTEROCOCCUS	NON-HEMOL.	HEMOL.
Lactose + Mannite + Salicin +	14	22	14		6	12	14	4
Lactose + Mannite + Salicin −	2	2	2		4	6	5	
Lactose + Mannite − Salicin −	10	6	10		8	18	10	4
Lactose − Mannite + Salicin +	6	10	6	2	4	8	8	4
Lactose − Mannite − Salicin +	2	16	2		10	20	6	
Lactose + Mannite − Salicin +	6	4	8		18	12	20	
Lactose − Mannite + Salicin −	2	0	0		2	6	0	
Lactose − Mannite − Salicin −	6	6	12		18	14	21	
Total	48	66	54	2	70	96	84	12

TABLE II

STREPTOCOCCUS

FERMENTABLE SUBSTANCE	VAGINA		CERVIX		FECES x		PHARYNX =	
	NUMBER STRAINS	PER CENT	NUMBER STRAINS	PER CENT	NUMBER STRAINS	PER CENT	NUMBER STRAINS	PER CENT
Mannite positive	24	42.86	35	36.46	239	76.0	24	14.0
Mannite negative	32	57.14	16	63.54	84	24.0	150	86.0

x Oppenheim[9]
= Arnold[2]

TABLE III

MANNITE FERMENTATION

	NEGATIVE		POSITIVE	
	NO. STRAINS	PER CENT	NO. STRAINS	PER CENT
Staphylococcus				
Vagina	24	50.0	24	50.0
Cervix	54	77.15	16	22.85
Enterococcus				
Vagina	32	48.5	34	51.5
Cervix	64	66.66	32	33.33
Streptococcus				
Vagina	32	57.14	24	42.86
Cervix	61	63.54	35	36.46

TABLE IV

	VAGINA				CERVIX			
	NON-PATHOGENIC		PATHOGENIC		NON-PATHOGENIC		PATHOGENIC	
	NO. STRAINS	PER CENT	NO. STRAINS	PER CENT	NO. STRAINS	PER CENT	NO. STRAINS	PER CENT
Staphylococcus	10	83.4	2	16.6	6	75.0	2	25.0
Streptococcus	24	75.0	8	25.	16	61.5	10	38.5
Enterococcus	9	75.0	3	25.	17	60.8	11	39.2

mannite fermenting power than are the cervical strains; the pharyngeal strains are merely given to show the other extreme in mannite fermentation.

In Table III, the mannite fermentations of the vaginal and cervical strains of staphylococcus, the so-called enterococcus and the streptococcus, are compared. Here it will be noticed that all of the vaginal flora of the Gram-positive coccus group ferment mannite more than those of the corresponding cervical flora. The limiting hydrogen-ion concentration for the three groups of Gram-positive cocci studied, did not differ markedly from one another. They varied from P_H 4.5 to P_H 5.6. The vaginal flora (170 strains in all) did not differ markedly from the cervical flora (262 strains) in this respect. The average H-ion concentration that limited growth was P_H 5.0. Table IV gives the results of the pathogenicity experiments with mice.

DISCUSSION

Avery and Cullen,[10] Brown[11] and Arnold[3] and others have divided the streptococci into human and bovine types by the limiting H-ion concentration. The human type is more sensitive to an acid environment and ceases to grow

at a H-ion concentration of P_H 4.8 to 5.4. The bovine type, on the other hand, is not so sensitive and the limiting H-ion concentration necessary to inhibit growth was found to vary from P_H 4.3 to 4.5. Brody and Arnold[12] have shown that there is no relationship between the limiting H-ion concentration of streptococci and their pathogenicity for mice. We suggested that the difference between the so-called human and bovine types was merely one of environmental conditions of growth. Streptococci isolated from areas in contact with an inflammatory exudation were of the human type; the bovine types came from areas not associated with exudation.

This suggestion is further confirmed as a result of the experiments reported in this paper. The total Gram-positive cocci isolated from the vaginal tract was 170, from the cervical 262. The average limiting H-ion concentration was P_H 5.0, the variations were between P_H 4.7 and 5.6. In other words, they were all within the human or pathogenic range. This we think was a result of the contact of these strains with the exudation present in the cervix and vagina of the cases studied. The bacteria found in the cervical canal are an extension upwards of those in the vaginal tract. The vaginal bacterial flora resemble in many respects certain fecal types. The Gram-positive cocci of the cervical canal differ from those found in the vaginal tract. There is a gradual loss of the power to ferment mannite, so typical of strains of fecal origin, and they become more pathogenic for mice. Both of these differences are probably due to the more parasitic mode of existence of the microorganism in the cervical canal under the condition found in our cases.

We have used only a few strains for our pathogenicity experiments and for this reason we do not place much value upon information gained by studying such a small number of cocci picked at random. We feel that a more detailed study of the pathology, etc., of the clinical cases from which the strains were isolated would be necessary before drawing definite conclusions as to our pathogenicity experiments.

SUMMARY

The Gram-positive cocci of the vaginal tract resemble closely the corresponding cocci of fecal origin. Those cocci present in the cervical canal of the uterus under various pathologic conditions, resemble those of vaginal origin. With gradual extension of the fecal types up through the vagina to the cervix, certain biologic changes take place: the power to ferment mannite is gradually decreased and an increased pathogenicity for mice is probably acquired. The differences in the limiting H-ion concentration of Gram-positive cocci is not an index to their pathogenicity, but those most sensitive to the hydrogen-ion concentration (P_H 4.8 to 5.4) have recently been in contact with an inflammatory exudate.

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LIVER FUNCTION IN VARIOUS DISEASES AS TESTED BY THE ADMINISTRATION OF LEVULOSE*

By Reuben Finkelstein, M.D., and Max Dannenberg, M.D., Brooklyn, N. Y.

IT is a well-known fact that the liver function is disturbed in many diseases both in acute and chronic conditions. Very often this disturbance in function is due to permanent pathology as is found at autopsy. In most cases the pathology is not severe enough or does not become permanent and the function returns to normal after the disease subsides. After pneumonia or influenza, permanent destruction of liver tissue is seldom found, although during the course of these diseases, its functions may be greatly disturbed.

It is evident that it would be very difficult to test each one of the numerous liver functions. We feel that this may be unnecessary in that disturbance of one function may be sufficient evidence of some degree of disturbance of all functions of the liver. It is hard to imagine that the ureagenic function of the liver will be altered without some change at the same time in its glycogenic function. The cause of these changes evidently is due to the diminished activity of the cells, which will affect all of its functions.

One of the most important functions of the liver is its glycogenic function, the conversion and storage of glycogen.

Since 1875 various investigators have sought to prove disturbed liver function through the ingestion of sugar. In that year Colrat[1] suggested the ingestion of 150 to 200 grams of cane sugar with subsequent examination of the urine. He found glycosuria in many conditions especially neurosis, hyperthyroidism, etc. He attributed this glycosuria to disturbed glycogenic function of the liver. The objection to this test is that saccharose is first converted into glucose in the alimentary tract. The rate of conversion varies in different people thereby introducing an unknown factor.

Later, glucose was used with the idea that no conversion would be necessary. But other organs besides the liver convert and store glycogen. This was proved definitely by Sachs[2] in 1899. He removed the livers of frogs and observed the tolerance to various sugars. The only sugar not used up was levulose. All the other sugars were converted into glycogen, thus proving that other organs besides the liver have the power of conversion and storage of glycogen.

Taking the above experiment into consideration, i.e., that levulose was

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TABLE I

CASES IN WHICH LEVULOSE FUNCTION TEST WAS PERFORMED

NO.	NAME	FASTING	½ HOUR	1 HOUR	2 HOURS	DIAGNOSIS
1	M.M.	.080	.083	.083	.078	Fibrosis of Lung
2	M.G.	.084	.120	.140	.106	Bronchial Asthma
3	J.K.	.080	.083	.083	.084	Gastric Ulcer
4	M.S.	.116	.122	.125	.132	Acute Cholecystitis
5	H.G.	.086	.088	.086	.070	Arthritis
6	J.R.	.055	.070	.070	.084	Influenza
7	I.S.	.076	.076	.060	.062	Duodenal Ulcer
8	L.T.	.076	.076	.075	.075	Pneumonia
9	J.R.	.109	.090	.083	.084	Lung Abscess
10	L.K.	.075	.075	.068	.064	Myocarditis
11	F.R.	.092	.100	.125	.150	Arthritis
12	M.R.	.083	.100	.108	.125	Pulmonary Tuberculosis
13	N.O.	.060	.160	.140	.100	Gastroduodenitis with Jaundice
14	J.G.	.087	.120	.107	.100	Chr. Cholecystitis
15	B.S.	.100	.120	.115	.106	Hydropneumothorax
16	B.K.	.106	.130	.130	.120	Chr. Myocarditis
17	Y.M.	.080	.125	.133	.100	Bronchial Asthma
18	G.A.	.100	.160	.160	.162	Influenza
19	T.M.	.150	.200	.250	.187	Lobar Pneumonia
20	S.R.	.100	.150	.160	137	Myocarditis
21	A.H.	.105	.110	.125	.200	Carcinoma Pancreas
22	P.S.	.138	.145	.166	.200	Chr. Cholecystitis
23	I.G.	.150	.155	.166	.200	Chr. Val. Disease with Ascites
24	R.R.	.125	.150	.194	.143	Cholecystitis
25	A.S.	.080	.080	.082	.084	Myocarditis
26	T.F.	.135	.130	.135	.135	Arteriosclerosis with Nephritis
27	J.F.	.140	.160	.135	.140	Arteriosclerosis
28	F.B.	.110	.100	.110		Chronic Nephritis
29	P.S.	.105	.105	.110		Chr. Appendicitis
30	H.A.	.100	.090	.090		Acute Tonsillitis
31	H.S.	.150	.150	.155		Arteriosclerosis with Nephritis
32	E.D.	.125	.150	.140	.135	Chr. Cholecystitis
33	R.L.	.110	.110	.100		Subacute Appendicitis
34	F.R.	.120	.110	.110		Pelvic Inflammation
35	B.J.	.110	.108	.110		Ulcerated Cervix
36	E.F.	.110	.135	.130	.120	Chr. Myocarditis
37	H.L.	.130	.130	.125		Carcinoma Lung
38	F.G.	.140	.135	.135		Arteriosclerosis with Nephritis

the only sugar not converted and stored as glycogen by the liverless frogs, Strauss[3] in 1901, formulated a new test for liver deficiency. He gave 100 grams of levulose in weak tea on a fasting stomach and examined the urine. No food was given after the previous evening meal. At 6 A.M. he obtained a normal urine, then gave the levulose. The urine was examined for levulose at hourly intervals for four hours. He found 76 per cent of negative tests in clinically normal liver cases, and positive in 83 per cent of clinically abnormal liver cases. The presence of a sufficiently large number of positive reactions in normal cases and negative reactions in abnormal cases is final proof that this test is not conclusive.

Many investigators have worked on this test and have given levulose in various quantities, but in all cases only the urine was examined at varying intervals. The great fault was that often too much levulose was given and an alimentary levulosuria resulted. Again the permeability of the kidneys to levulose varies with different individuals. This test also soon fell into disuse because of its uncertainty.

In 1921, McLean and De Wesselow[4] studied blood sugar curves of normal adults and that of diabetics after the ingestion of various sugars, such as glucose, levulose, saccharose, galactose, etc. All these sugars, excepting levulose, provoked a rise in the blood sugar. As little as 25 grams of these sugars provoked a maximal rise. Larger doses were incapable of increasing the level, merely tending to prolong the time during which this level was main-

TABLE II

DISEASES IN WHICH LEVULOSE FUNCTION TEST WAS FOUND POSITIVE

NO.		BLOOD SUGAR FASTING STOMACH	½ HOUR AFTER INGESTION 50 GRAMS LEVULOSE	1 HOUR AFTER INGESTION	2 HOURS AFTER INGESTION
2	Bronchial Asthma	.084	.120	.140	.106
17	Bronchial Asthma	.080	.125	.133	.100
4	Acute Cholecystitis	.116	.122	.125	.132
11	Arthritis	.092	.100	.125	.150
6	Influenza	.055	.070	.070	.084
18	Influenza	.100	.160	.160	.162
19	Pneumonia	.150	.200	.250	.185
20	Myocarditis, Enlarged Liver	.100	.150	.160	.137
36	Myocarditis, Enlarged Liver	.110	.135	.130	.120
16	Myocarditis, Enlarged Liver	.100	.130	.130	.120
12	Pulmonary Tuberculosis	.083	.100	.108	.125
13	Gastroduodenitis with Jaundice	.060	.160	.140	.100
14	Chr. Cholecystitis	.087	.120	.107	.100
22	Chr. Cholecystitis	.138	.145	.166	.200
24	Chr. Cholecystitis	.125	.150	.194	.143
32	Chr. Cholecystitis	.125	.150	.140	.135
15	Hydropneumothorax	.100	.120	.115	.106
21	Carcinoma of Pancreas	.105	.110	.125	.200
23	Chr. Val. Disease with Ascites	.150	.155	.166	.200

TABLE III

DISEASES IN WHICH LEVULOSE LIVER FUNCTION TEST WAS FOUND NEGATIVE

NO.		BLOOD SUGAR FASTING STOMACH	½ HOUR AFTER INGESTION 50 GRAMS LEVULOSE	1 HOUR AFTER INGESTION	2 HOURS AFTER INGESTION
1	Fibrosis Lung	.080	.083	.080	.078
3	Gastroduodenal Ulcer	.080	.083	.083	.084
7	Gastroduodenal Ulcer	.076	.076	.060	.062
5	Arthritis	.086	.088	.086	.070
8	Pneumonia	.076	.076	.075	.075
9	Lung Abscess	.109	.090	.083	.084
25	Myocarditis	.080	.080	.082	.084
10	Myocarditis	.075	.075	.068	.074
26	Arteriosclerosis, Nephritis	.135	.130	.135	.135
27	Arteriosclerosis, Nephritis	.140	.160	.135	.140
31	Arteriosclerosis, Nephritis	.150	.150	.155	.155
38	Arteriosclerosis, Nephritis	.140	.135	.135	
28	Chr. Nephritis	.110	.100	.110	
29	Chr. Appendicitis	.105	.105	.110	
30	Acute Follicular Tonsillitis	.100	.090	.090	
33	Subacute Appendicitis	.110	.110	.100	
34	Pelvic Inflammation	.120	.110	.110	
35	Ulcerated Cervix	.110	.108	.110	
37	Carcinoma Lung	.130	.130	.125	

tained. Levulose on the contrary, had no effect on the blood sugar in persons with normal livers. This proves the original experiment of Sachs in 1899 as mentioned above.

Spence and Brett[5] later, found that the ingestion of 50 grams of levulose will show no rise in the blood sugar. More than 50 grams may cause an alimentary levulosuria because even a normal liver has a limit of storage and all levulose above this limit will be spilt over into the blood and excreted by the kidneys. The rationale of this test therefore depends upon the fact that the normal liver is able to convert and store 50 grams of levulose given at one time. If the liver is in any way damaged the entire amount would not be taken up by its cells and some levulose would appear in the blood.

We have performed the levulose test on thirty-eight consecutive patients admitted to hospital. No food was given after the evening meal. Next morning, the patient still fasting, blood was taken and its sugar content determined. Fifty grams of levulose dissolved in water was administered. The blood sugar was again determined at half hour intervals for two hours. The blood in cases one to twenty-six was tested by the Benedict method; in cases twenty-seven to thirty-eight by the Epstein method. The positive and negative results are about equally divided. The nineteen cases in which no increase in blood sugar was observed represent fourteen different diseases. The other nineteen cases representing twelve various diseases gave an elevated blood sugar curve for a period of at least two hours.

While the series is rather small for conclusive deductions, it will be noted that the test was positive in acute and chronic gall bladder infections, (five cases); in icterus, in carcinoma of the pancreas, and in chronic passive congestion of the liver (four cases).

The curve was also positive in a few other conditions in which there was no other clinical evidence of hepatic injury.

If we assume that the abnormal blood sugar curves observed are indicative of altered liver function, it would appear that such alteration occurs in some cases in which we have, as a rule, not suspected hepatic damage.

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OBSERVATIONS ON THE RELATION OF URINARY P_H TO SALT AND WATER METABOLISM IN A CASE OF NEPHROSIS*

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THERE is such a voluminous literature dealing with nephrosis, or chronic parenchymatous nephritis, and there are so many case reports, that one hesitates to add still another to the list. This case, however, is probably worthy of consideration because of the length of time over which observations were continued and the apparent relationship of the urinary P_H and salt and water metabolism.

As is well known, the views of equally competent observers are diametrically opposed when the salt metabolism is investigated. Widal, Abrami, Vallery Radot[1] and their school believe that the kidney is the determining factor in salt retention; that there is a blood salt retention whenever the kidney is unable to excrete the amount of salt ingested, and that by dialysis the excess salt is distributed to the tissues, thus increasing their osmotic power, and causing edema.

Martin H. Fischer[2] believes that the kidney *per se* is not primarily at fault. He teaches that, because of certain changes in the cells and fluid tissues of the body, all of which are colloidal in nature, there is an increased affinity for water and neutral salts, so that these are taken up from any available source. The kidney is involved in this process only as one organ in a widely spread pathologic change. The increaesd salt content of the tissues is a concomitant change; a result of the factors responsible for the tissue changes and not the cause of the edema. There are three types of materials which experimentally can produce these changes. They are acids, alkalies, and amines. Acids are constantly formed in the body as a result of metabolism, and there are finely adjusted mechanisms for their neutralization so that the normal body alkalinity may be preserved. There are, however, many conditions in which there is a profound disturbance of this regulatory mechanism which may be confined to some localized area or may be widespread. In cardiac failure, for example, there is stasis first in the feet and legs as these are farthest from the heart. This stasis prevents a normal supply of oxygen to the tissues with the result that the metabolites are incompletely oxidized and lactic acid is produced in large quantities. This combines with the cell protoplasm, increasing their acid content or perhaps in severe cases their P_H. These cells then swell, absorbing water from the vessels near by. Alkali in excess may produce similar effects but this condition is not met with except in cases poisoned by alkalies. The amines, which act similarly, are the most

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frequent offenders. Most bacterial toxins are amines, and many are formed when any protein material is broken down.

While these views of Fischer were condemned by many observers when they were first announced, the pendulum of thought is gradually swinging in the direction of their general confirmation. Most clinicians now regard the parenchymatous nephritides as generalized toxemias and Marriott[3] recently stated that Claussen had isolated a toxic substance from the urine and blood of these cases, which would produce salt retention and edema when injected into animals. He stressed the importance of removing all foci of infection and reported cases in which complete cures were obtained by these methods. Eppinger[4] also states that the chief difficulty in the edemata lies in the subcutaneous tissues, and not in the kidneys.

Recently Aldrich and McClure[5] reported their observations on the intracutaneous salt solution disappearance time. Briefly, they found that in individuals with edema, salt solution injected into the skin disappeared in from a few seconds to a few minutes, while in normal individuals it required sixty minutes. They were able to predict clinical improvement with loss of edema whenever the disappearance time increased. These observations point to increased water-holding power of tissue as the cause of edema.

The patient M. L. R., aged twenty-six, was admitted to the medical service of Mount Sinai Hospital from the Out-Patient Department, on February 3, 1924, for a study of salt metabolism in relation to edema. He had previously been hospitalized here from May 15, 1923, until July 7, 1923. On his first admission he gave the following history:

Early in January he noticed a marked swelling of the left submaxillary regions for which he consulted a nose and throat specialist, but for which no local cause could be found. About one week later his feet and legs began to swell. He consulted a physician who told him he had kidney trouble and advised him to take things easy and to restrict his fluid intake. Rest and fluid restriction at home enabled him to return to work, but in ten days the edema recurred and he entered the hospital.

The family history was irrelevant. The previous history stated that he had had some of the diseases of childhood, which ones he did not know, but that there was apparently complete recovery from them. No acute infection preceded the present illness, nor did the patient remember any serious illness. Physical examination revealed a well-developed white male of twenty-five years with slight edema of the face and shins. The eyes, nose, mouth, throat, thorax and abdomen showed no abnormalities. The reflexes were normal. The blood pressure was 140/80. Laboratory examination revealed:

Urine: total volume 1000 c.c., acid, and specific gravity 1.025. Albumin in large amount, sugar negative. Microscopically, a few white blood cells and an occasional cast were found.

Phenolsulphonephthalein test showed 65 per cent in two hours, 70 per cent in three hours. The Mosenthal test showed no fixation of specific gravity. It varied between 1.005 and 1.015 with a night specific gravity of 1.010 and a volume of 480 c.c.

Blood count showed hemoglobin to be 70 per cent, white blood cells 7,800.

Nonprotein nitrogen	32 milligrams per 100 c.c.
Uric acid nitrogen	17
Urea nitrogen	2.4
Creatinine	1.5
Glucose	100
Plasma chlorides	626

Dietary management, reduction of salt and water, together with the use of Novasurol produced marked improvement and he was discharged to the Out-Patient Department on July 7, 1923.

In the dispensary he was instructed to limit his fluid intake to 1,000 c.c. daily and to eat a salt-poor diet. This he faithfully carried out, but in spite of good care, the edema recurred and he was re-admitted to the hospital.

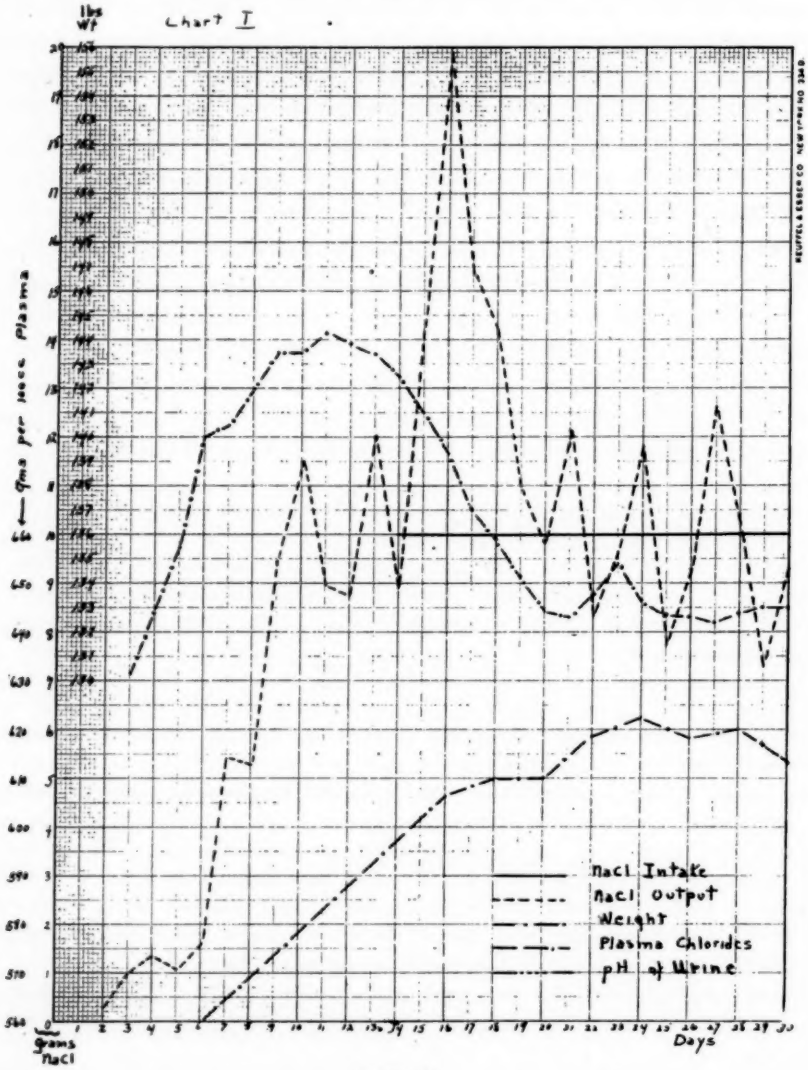

Chart I.

At the time of the second admission the urinary output was 500 c.c. and contained only 0.2 grams of sodium chloride. The plasma chlorides measured 530 mg. per 100 c.c. showing that he had been on a very good salt-free diet. The other findings were comparable to the previous admission. He was placed on a full house diet for twelve days and was then given a full diet containing ten grams of sodium chloride daily.

Records were made of the exact sodium chloride intake, the urinary volume, the percentage of salt output, the total salt output, the weight, the plasma chlorides, and urinary P_H. The charts show in a graphic way the re-

sults obtained. Chart I shows the gradual gain in weight produced when a full house diet, the salt content of which was not known, was substituted for the salt-free diet which he had been taking for several months. This rise was paralleled by a rise in total sodium chloride output which reached 12 grams. A diet containing exactly 10 grams of salt was then begun. There was a sharp rise in total sodium chloride excretion with a sharp loss of weight. The

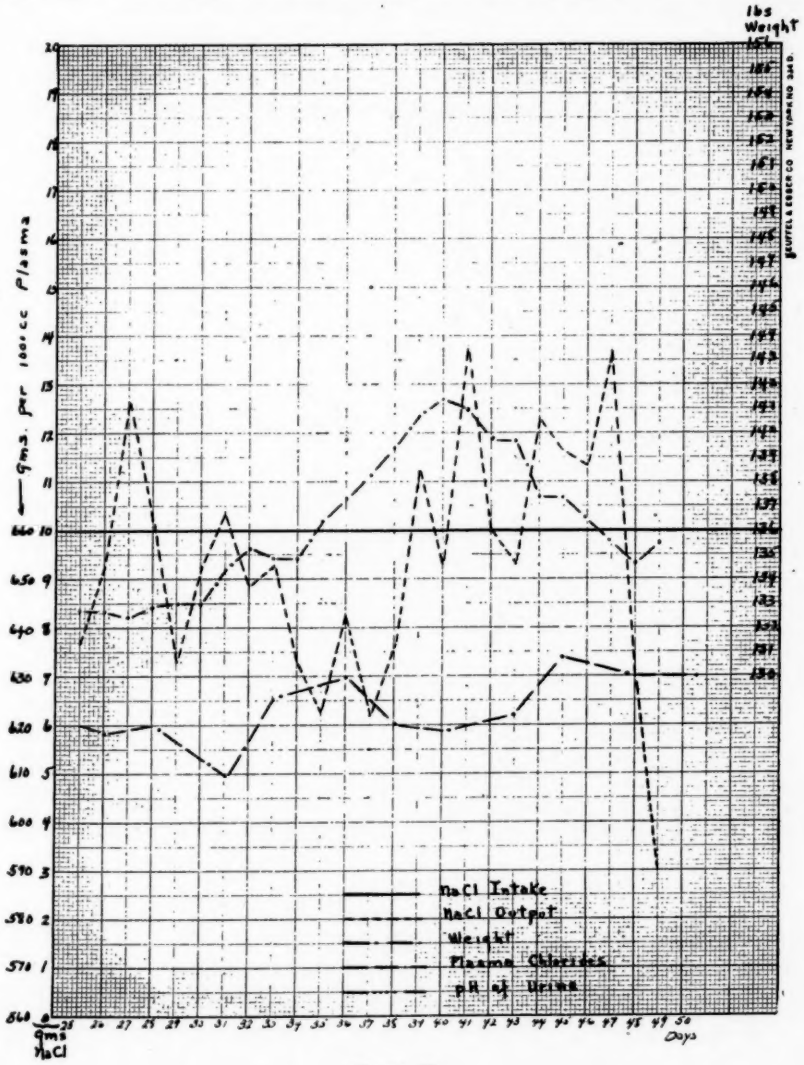

Chart II.

salt excretion and intake then practically balanced each other but the weight fluctuated as much as two pounds. The plasma chlorides rose gradually from 560 to 620 mg. per 100 c.c.

Chart II shows remarkable variatons in weight and in sodium chloride excretion despite the stable intake of 10 grams of sodium chloride. The weight fluctuated a maximum of nine pounds and sodium chloride output a maximum of 7 grams. Obviously there must be some factors other than salt

intake which determine the salt output and the weight. In examining the urine it was noted that during the sharp rises in weight with decreased salt output, the urine was highly acid; accordingly the P_H of every specimen voided was determined colorimetrically and the average for the day plotted in relation to the other curves. Charts III and IV show the marked urinary acidity coincidental with the diminished salt output and the gains in weight. The urine P_H was between 6.6 and 7.0, the chloride output between 11.5 and 13.6 grams, and the weight 134½ pounds. There was then an abrupt decrease in urinary P_H with a marked decrease in the percentage of sodium chloride output and a sharp drop in the total number of grams of sodium chloride. When

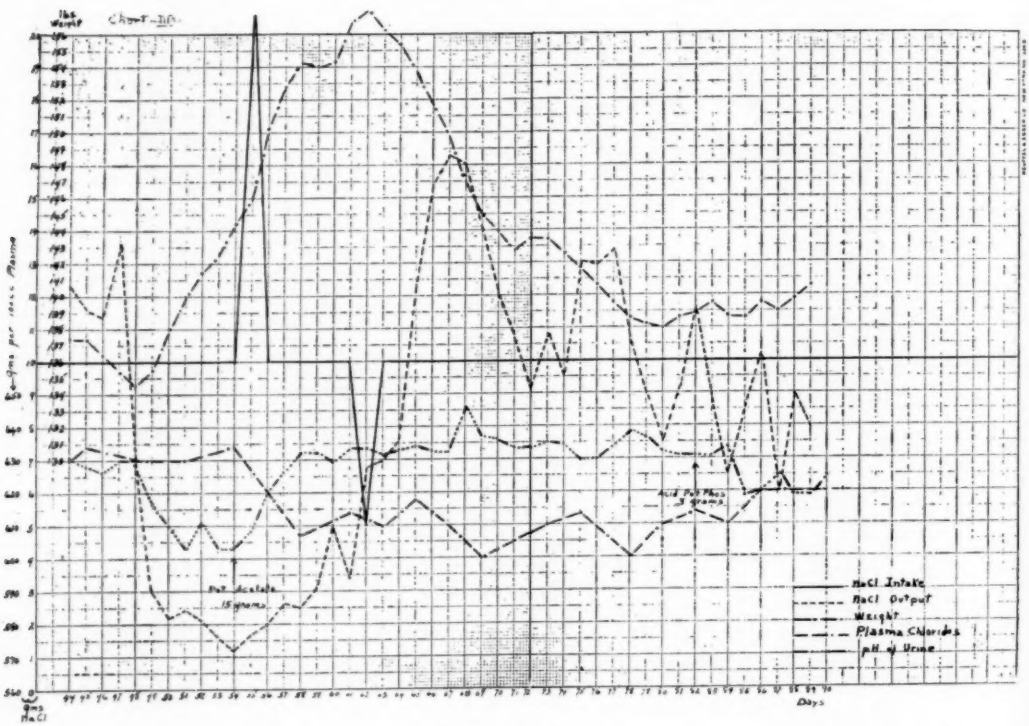

Chart III.

the urinary P_H reached 4.4, the volume was 330 c.c., the sodium chloride 0.4 per cent and the total sodium chloride fell to 1.2 grams.

At this point 15 grams of potassium acetate were administered daily in an attempt to reduce the urinary acidity. It required seven days to bring the urine to a neutral reaction. As the acidity lessened the first noted change was a marked increase in the per cent of sodium chloride excreted; the second, an increase in urinary volume and of course a corresponding increase in total sodium chloride excreted. The weight continued to rise, reaching a maximum of 158 pounds before the total sodium chloride excretion reached 10 grams and balanced the intake. In other words it required six days after the chloride output was stimulated for the step-like rise described by Vallery Radot to take place, to effect a balance between intake and output. While the urinary P_H was maintained at 7.0 or above there was a progressive loss of fluid until

138 pounds weight was reached. At this point the urinary P_H was reduced to 6.0 and a slight gain in weight was recorded. The weight of 138 pounds represented a clinical condition similar to that on admission. There was no edema of the face, but slight pitting over the shins. There had been a physiologic gain of weight. During these observations, estimations of the carbon dioxide combining power of the plasma were carried out every other day. There were no significant variations.

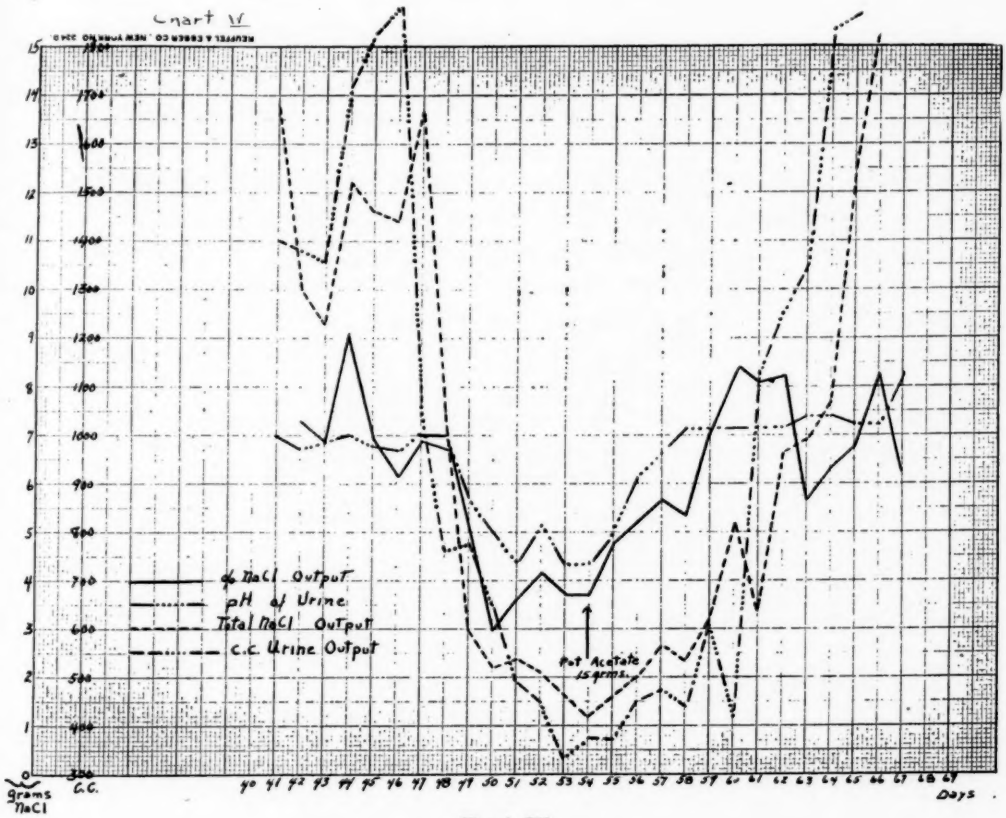

Chart IV.

SUMMARY

Records of the urinary volume, per cent chloride output, total sodium chloride output, urinary P_H, plasma chlorides and weight were kept for 110 days in a case of nephrosis on a diet containing 10 grams of sodium chloride daily.

With a rise in weight there was a chloride retention, diminished water output, and a heightened acidity of the urine. Administration of alkalies to render the urine neutral was accompanied by a sharp increase in chloride output and loss of edema. Administration of acid potassium phosphate reversed the process and was followed by a gain in weight.

I wish to express my thanks to Dr. S. S. Berger for his kindness in permitting me to make the observations on this case.

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ENDO'S MEDIUM*

By C. C. Young, D.P.H., and M. S. Marshall, M.A., Lansing, Michigan

AFTER a special culture medium has been developed for a particular use, modifications begin. The medium is tried for purposes for which it was not intended, variations are made which simplify or improve it for the original use, and changes creep in the technic of preparation or in the formulae which are often insidious. The principle is certainly commendable, but the result is that the new worker is lost in the variations, and the more experienced worker is unsettled as to which variations are preferable, allowable, and advantageous. It is to be hoped that the manual of culture media in preparation under the auspices of the Society of American Bacteriologists, under the guidance of Dr. Buchanan, and with the help of the Digestive Ferments Company, will have a tendency to stabilize such tendencies and overcome such duplication of work.

It is the purpose of the present paper to summarize briefly some of the chief Endo medium variations, and to present experimental data which point out evidence on the whole confirming some points brought out by other authors, although not agreeing with all reported work.

In Table I are charted the formulae for the more common "Endo" media.

There is agreement on the amount of peptone and the amount of lactose throughout; in all other ingredients there is variation. Perhaps the most used variation from the original Endo medium is Kendall's modification, which was based on the necessity for a rapid diagnosis of bacillary dysentery; Endo's original medium was made for typhoid diagnosis.

The significant points may be given as:

1. Variation in the percentage of agar: increase in percentage decreases the *rate* of diffusion of the color-changing substances. Hence a low percentage is advantageous for rapid work, but dangerous if used where the time of reading is not under careful control. Variation is useless without careful P_H control, for a neutral medium will change color more rapidly than an alkaline medium.

2. Use of salt: not of material importance, unless there be some slight effect upon agglutinative properties of the organisms, which is doubtful.

*Michigan Department of Health.

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TABLE I

SUMMARY OF "ENDO" MEDIA

#	ENDO[1]	KENDALL[2]	K. AND D.[3]	R. AND R.[5]	L.[8]	P. AND W.[4a]	STITT[21]	APHA[22]
Agar	3.0	1.5	1.5	2.5	1.5-3.0	2.5	3.0	3.0
Peptone	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.5	–	0.5	–	–	–	0.5	–
Lactose	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Beef ext.	–	0.3	0.3 or	0.5	–	0.5	0.5	0.5
Beef inf.	+	–	±	–	–	–	–	–
Fuchsin x	0.5	0.09	0.25	0.5	0.5	0.5	0.6 b	0.5
Na_2SO_3 y	2.5	0.9	–	–	2.5	–	2.0 b	2.5
$NaHSO_3$ z	–	–	0.5	1.0	–	1.0	–	–
Px	7.8 c	7.0 d	7.6-7.8	8.0 e	no adj.	8.2	8.0 f	"as required"
K_2HPO_4	–	–	–	–	0.2-0.5	–	–	–

\# Figures represent percentages.
x Saturated alcoholic solution.
y 10 per cent aqueous, fresh.
z 10 per cent aqueous, fresh.
a As Robinson and Rettger,[5] except for PH.
b 6 drops per 100 c.c.; 20 drops per 100 c.c.
c Neutral to litmus + 1 per cent of a 10 per cent Na_2CO_3.
d Just alkaline to litmus.
e +0.1 per cent; 6.8 + 1 per cent of a 10 per cent Na_2CO_3.
f 0.0 or + 0.2 per cent.

3. Use of beef extract: most authors have adopted the more convenient meat extract instead of using fresh meat infusion. This point is questionable, since we are dealing in a diagnostic laboratory with somewhat delicate pathogenic forms not of optimum viability.

4. Variations in amounts of fuchsin and sodium sulphite. Much obviously depends on these quantities.

5. The P_H of the media: this has been stated to be important,[3, etc.] and should with the advent of P_H control methods be consistently established.

Harding and Ostenberg,[19] DeBord,[11] and Kahn,[14] have given experimental evidence of the nature of the Endo reaction. The color of the fuchsin-sulphite indicator is restored by organic acids and aldehydes, and by acetone and alcohol. These may be produced in different amounts and in different ratios by different organisms. It has been the general experience by those who have used Endo's medium in routine examinations of feces that the identical medium will present somewhat different aspects when inoculated with different fecal flora. The ideal Endo medium will differentiate the typhoid, paratyphoid, or dysentery cultures in the presence either of weak or of violent color-producers.

Experiments were made with a view to checking the use of beef extract and beef infusion, the amounts and ratio of basic fuchsin and sodium sulphite, and the P_H. Preliminary experiments were made with media containing beef extract or beef infusion, with saturated alcoholic basic fuchsin in varying amounts up to 0.5 per cent, each combined with varying amounts of sodium sulphite up to 3.5 per cent of a 10 per cent fresh aqueous solution. These combinations were tried at P_H values from 6.5 to 7.7. Four feces were inoculated to plates of each combination of variables, using *B. typhosus* and *B. dysenteriae* cultures in dilute suspension in the feces.

The results showed that Endo's original formula gave the largest typical colonies, the least diffusion, and the best differentiation. This medium has a

beef infusion agar base, uses 0.5 per cent fuchsin solution with 2.5 per cent sodium sulphite solution, and has a P_H of from 7.5 to 7.7.

To secure unbiased results from these media from routine work, six different combinations of fuchsin and sulphite were outlined, from 0.05 per cent to 1.00 per cent fuchsin solution, and from 0.50 to 5.00 per cent sodium sulphite solution, all at a P_H of 7.5 to 7.7, and with either a beef extract or a beef infusion base. These were supplied under a code number by the media division of the laboratory to the diagnostic division for a period exceeding one month,* the latter reporting their results daily. Freshly isolated *B. typhosus* and *B. dysenteriae* were used in control plates throughout.

The results were in favor of beef infusion agar, with 0.2 to 0.5 per cent fuchsin solution and 1.0 to 2.5 per cent sodium sulphite solution, the two in a respective ratio of 1:5.

Different concentrations of a 1:5 fuchsin sulphite medium, representing the above concentrations, were added to an Endo base without lactose. Pure cultures of *B. coli, B. typhosus,* and *B. dysenteriae,* all freshly isolated from feces, were inoculated to the plates.

TABLE II

EFFECT OF DECOLORIZED FUCHSIN ON SIZE OF COLONY

Basic fuchsin sol.	0.1	0.3	0.5	1.0	1.5 per cent
Sodium sulphite sol.	0.5	1.5	2.5	5.0	7.5 per cent
B. coli, 1st trial	1.7*	2.0	2.0	1.7	2.0 mm.
2nd trial	2.5	2.5	2.0	2.0	2.0
B. typhosus, 1st trial	1.0	0.8	0.5	0.8	0.5
2nd trial	2.0	1.5	1.0	0.7	0.5
B. dysenteriae, 1st trial	1.0	1.5	1.0	0.7	0.5
2nd trial	2.5	2.0	1.5	0.7	0.0

*Diameter of colonies at 18 hours in mm.

The bacteriostatic action of the fuchsin is evident on *B. typhosus* and *B. dysenteriae,* but we have seen no evidence that the original Endo amounts of fuchsin-sulphite mixture are seriously inhibitive, although a slightly smaller amount has given satisfactory differentiation and growth.

It has been objected that fuchsin and sulphite vary in different lots of chemicals; the procedure of Kastle and Elvove, titrating fuchsin with sulphite to a maximum sensitivity with formaldehyde, would overcome such difficulty. There has been, however, no cause for worry on this point in our experience.

SUMMARY

A brief summary of the most common Endo media in use is given, with references.

A brief write-up of an extended series of feces examinations by Endo media of varying meat base, P_H, and fuchsin-sulphite amounts and ratio is given.

The conclusions of Gilbert and Coleman based on 4,552 examinations, namely, that the original Endo medium is as satisfactory as any for typhoid,

*NOTE: 1280 feces examinations were made at this laboratory during the fiscal year 1923-1924.

paratyphoid, and dysentery diagnosis, and more satisfactory than some, is endorsed. In justice to the various writers discussed, further conclusions will be left to the reader, as each writer has directed his attention to his own problems.

Acknowledgment is made to Miss Lucy Dell Henry, who handled all of the media and a considerable part of the experimental work.

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TREATMENT OF NASAL DIPHTHERIA CARRIERS*

By Clara Israeli, M.D., Manchester, N. H.

THE carrier problem in infectious diseases is a difficult one, because clinical symptoms are lacking or are of such mild character that a correct diagnosis is not made. It is only after cultural study that a diagnosis is made. This is especially true of diphtheria and typhoid carriers.

In the case of throat diphtheria carriers, tonsillectomy has been resorted to, but this practice is not applicable to nasal diphtheria carriers. Treatment with mild antiseptic douches and sprays in nasal diphtheria cases has not given satisfactory results. There are rhinologists who are so radical as to state that no treatment is of avail.

In diphtheria we are dealing with a local infection of the mucous membrane. Other organisms, having already made the mucous membranes vulnerable or actually diseased, favor the growth of diphtheria bacilli. The latter cause excoriation of the mucous membrane and bleeding. With such a pathologic condition, it seemed to us that if the mucous membrane could be healed with some substance which would at the same time destroy the organisms, the results would be favorable. Since antiseptic solutions have not filled the need, it seemed to us that ointment might be worthy of trial. For this purpose a ten per cent ichthyol ointment was used. It is a mild antiseptic, allays inflammation, aids in destroying the microorganisms and thus favors healing of the mucous membrane.

The first case treated was a child two years old, who had a nasal discharge and an alternate daily positive culture. To establish the site of infection, cultures were taken from the nose, throat and ears. There was a discharge from the ears. The nasal culture alone showed diphtheria organisms.

The method used was as follows: A piece of absorbent cotton was wound tightly on the end of an applicator, and as much of the ointment as would hold on the end of the swab was carried gently all the way into the nasal cavity, entering the nasopharynx; another swab is used in the same way in the other nasal orifice.

This method of treatment was carried out five consecutive days. Nasal discharges ceased. Treatment was omitted for two days. A culture was taken on the third day. Growth was scant, with a few staphylococci, but no diphtheria bacilli. Cultures were taken on three consecutive days, all giving negative results. The patient was discharged. A culture taken a month later gave negative results.

The next were two cases—sister and brother—admitted to the hospital,

*Read by title before the Third Annual Meeting of the American Society of Clinical Pathologists, Rochester, Minn., June 7, 1924.

	HOME	PRELIMINARY CULTURE	NO. OF DAYS TREATED	NO. OF DAYS OMITTED	CULTURE AFTER TREATMENT	RESULTS	SECOND WEEK TREATMENT	DAYS OMITTED	CULTURE	RESULTS	SECOND DAY CULTURE	THIRD DAY CULTURE	THIRD DAY	CULTURE ONE MONTH LATER	
1. R. D. 4 months	Some Time	Nose + and Throat −	5 days	2 days	Third day	Diphtheria almost pure culture	5 days	2 days	3rd.	Scant growth. Few diplococci. No diphtheria	Scant growth. No diphtheria	Growth scant. No diphtheria	Patient discharged	Negative for diphtheria	
2. J. Dr. Coburn's case	Some Time	Nose + and Throat −	5 days	2 days	Third day	Diphtheria	5 days	2 days	3rd.	Growth diphtheria	Treatment 5 days	Omitted 2 days	Culture Third day	Negative for Diphtheria	Second and third day cultures were negative for diphtheria

NO.	NAME OF PATIENT	HOSPITAL	PRELIMINARY CULTURE	NO. OF DAYS TREATED	NO. OF DAYS OMITTED	DAY OF CULTURE TAKEN	RESULTS	SECOND DAY CULTURE	THIRD DAY CULTURE	CULTURE ONE MONTH LATER	TOTAL NO. OF DAYS IN HOSPITAL
1.	W. K., 2 yrs.	2 wks.	Nose + Throat − Ears −	5 consecutive	2 days	Third day	Scant growth few diplococci. . No Diph.	Scant growth. No Diph.	Scant growth no diph. Patient discharged	Negative results	24 days
2.	A. Z.	On day admitted	Nose + Throat − Ears 0	5 days	2 days	Third day	Few diplococci. No Diph.	Few diplococci. No Diph.	No Diphtheria. Patient discharged		10 days
3.	M. Z.	On day admitted	Nose + Throat − Ears 0	5 days	2 days	Third day	Few diplococci. No Diph.	Few diplococci. No Diph.	No Diphtheria. Patient discharged		10 days
4.	L. G.	On day admitted	Throat − Nose + Ears 0	5 days	2 days	Third day	Few diplococci. No Diph.	Few diplococci. No Diph.	No Diphtheria. Patient discharged		9 days

having been isolated on culturing the school for diphtheria carriers. These two cases had a severe nasal discharge with bleeding, excoriated mucous membrane. Cultures taken from the nose and throat showed the nose alone to be the site of infection. There was no discharge from the ears, so cultures were omitted. Treatment was carried out five consecutive days. Nasal discharge ceased, the mucous membrane healing well. Treatment was omitted for two days. A culture was taken on the third day. Growth showed few staphylococci, no diphtheria bacilli. Cultures were taken on three successive days, all giving the same amount of growth, but no diphtheria organisms. The patients were discharged.

The next case was a child four months old which was cared for at home. The culture showed a persistent nasal diphtheria infection almost in pure culture. The people objected to quarantine and to having the physician take so many cultures. The health department sent a nurse to carry out the treatment according to the above method. At the end of the first week when the first culture was submitted to the laboratory, the growth was in almost pure culture, proving that treatment was not carried out faithfully. Another week was used in treating this case under specific directions, with ten per cent ichthyol ointment. At the end of the week a culture submitted to the laboratory, showed scanty growth, with a few diplococci, but no diphtheria bacilli. Cultures were taken on three successive days. The growth was the same. The patient was discharged and quarantine removed. A culture taken a month later gave negative results.

Several other cases were treated according to this method with satisfactory results. A few physicians who had private cases with persistent nasal diphtheria, have on our recommendation, used this method with satisfactory results. Some of the physicians submitted cultures to us for control while they had their patients treated. It was found that it required three weeks to a month to obtain as completely successful results as obtained by us.

The cases under our observation did not show any signs of sinusitis.

COMMENT

We present this short report because we have found the treatment successful. It is simple, inexpensive, and easily applied. As a rule the time is comparatively short, depending, of course, on the faithfulness of the applications. We reiterate this point, because, as we have indicated above, by our control work, it was shown, that the longest time required for successful results was in cases where treatment was haphazard. It is fair to say that treatment was not carried out by the physicians, but left to a nurse.

The cases treated by us, and those treated by other physicians have not so far as we have been able to determine, been a source of infection, either in their homes or in school. They would have again come under our notice as they did in the first place.

THE RELATION OF THE GALL BLADDER TO THE DEVELOPMENT OF JAUNDICE FOLLOWING OBSTRUCTION OF THE COMMON BILE DUCT*

By Frank C. Mann, M.D., and Jesse L. Bollman, M.D., Rochester, Minn.

IN our studies on the effect of total removal of the liver, we noted that jaundice developed in the hepatectomized animal. The urine secreted shortly after operation was bile-colored. Within six hours, the plasma became yellow, and the jaundice increased progressively until death. If animals lived sixteen hours or more a definite icteric tinge developed in the sclerotic and mucous membranes. At necropsy all the fatty tissues were a dirty yellow. This yellow pigment which accumulated in the urine, plasma and fat reacted positively to the accepted tests for bilirubin, thus definitely proving the liver is not essential for the formation of bile pigment.

A comparison of our data on the formation of bilirubin after hepatectomy, with certain observations on jaundice following occlusion of the common bile duct, showed that there was seemingly a discrepancy between the rate of development of jaundice after hepatectomy and after common bile duct obstruction. When the common bile duct of a dog was obstructed, a demonstrable amount of pigment did not appear in the blood for many hours, whereas when the liver was removed, the pigment appeared within a few hours. This seemed to imply that there was an increase in formation of bilirubin after hepatectomy, and might imply an increase in blood destruction. However, the red cells and hemoglobin did not show an increase in blood destruction after hepatectomy, but remained practically normal. In order to determine the reason for this discrepancy between the rate of accumulation of bilirubin in the blood following hepatectomy, and that following occlusion of the common bile duct, as well as to obtain data with regard to the rate of formation of bilirubin with the liver intact as compared with the rate after hepatectomy, a series of experiments was performed.

PREVIOUS WORK ON THE CONCENTRATING ACTIVITY OF THE GALL BLADDER

One of us has recently published a review of the literature on the functions of the gall bladder, so that literature related to the subject will only be discussed briefly. Kemp called attention to the difference between the character of the bile secreted by the liver and that found in the gall bladder, and to the inspissated character of the bile in the gall bladder. Bidder and Schmidt found, experimentally, that much fluid is absorbed from the bile in the gall bladder. Hohlweg demonstrated that by the gall bladder's absorption of water; it could concentrate the bile secreted during the intervals between digestion, so that the 40 c.c. of bile found in the gall bladder represented from 240 to 400 c.c. of bile from the liver. Rous and McMaster ligated the

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common bile duct of dogs, and the bile secreted by a portion of the liver went into the gall bladder, while that secreted by the remainder of the liver passed into the bile duct, to which a rubber balloon was attached. Control observations were made on the uniformity of the pigment content of the bile secreted from different portions of the liver. It was found that the pigment content of each cubic centimeter of bile in the gall bladder might be ten times greater than the bile secreted into the duct, showing that the bile in the gall bladder had been greatly concentrated. Afanassiew observed that jaundice developed much more rapidly in animals when the biliary tract was filled with wax than when the common duct alone was occluded. Bloom, in studying the rôle of the lymphatics in the absorption of bile pigment from the liver in cases of early obstructive jaundice, found that bilirubin appeared in the blood about two hours after ligation of the common bile duct and exclusion of the gall bladder, while positive tests for the pigment were obtained on the lymph from the same animal from forty-five to sixty-two minutes after ligation.

METHOD

All the experiments were performed on dogs; all operative procedures were performed under ether anesthesia, employing aseptic technic. In one group of animals the common bile duct was sectioned between clamps and doubly ligated; the remaining portion of the biliary tract was left intact. In the other group of animals the common bile duct was obstructed in the same manner, and the gall bladder was removed. Observations were made on the time of appearance and rate of accumulation of bile pigment in the plasma and urine of the two groups of animals. The van den Bergh method was employed for the quantitative estimation, as well as qualitative tests.

RESULTS

The results of the experiments are very striking (Fig. 1). Animals in which the common bile duct is obstructed and the gall bladder left intact do not have an appreciable amount of bile pigment in the urine for from thirty-six to forty-eight hours after operation; only a faint trace of bilirubin, if any, is found in the plasma at the end of twenty-four hours. The scleras do not develop an icteric tinge for from forty-eight to seventy-two hours. While the various animals differ slightly, it is usually forty-eight hours between the time of obstruction of the common bile duct, and the appearance of the first definite evidence that jaundice is developing. The subsequent development of icterus and the accumulation of bilirubin in the blood is also slow. Well defined icterus is usually not observed for more than forty-eight hours after the first appearance of bile pigment in the blood. When the gall bladder is removed at the same time the duct is ligated, the length of time between operations and the first appearance of jaundice is greatly decreased. Bile pigment appears in the urine and a definite test for bilirubin is obtained in the plasma within from three to six hours after the ligation of the common duct and the removal of the gall bladder. The amount of pigment in the plasma increases rapidly, and the jaundice is fully developed within twenty-four to forty-eight hours. Before the end of the first twenty-four hours the

sclerotics and mucous membranes are definitely icteric, and there is no question about the animal being jaundiced.

The great difference between the rate of the development of jaundice in animals in which the common bile duct is obstructed and those in which the gall bladder is removed and the same obstruction produced, is illustrated very clearly by comparing the evidence of icterus in the two series. The sclerotics and the mucous membranes in the animals of the first series appear normal for from three to four days after operation, while those of the second series are definitely icteric after twenty-four hours. The urine of the animals of the first series remains free from bile pigment for from thirty-six to forty-eight hours, while the second series contains large amounts within three to six hours, and is found in all urine passed after that time. Comparison of

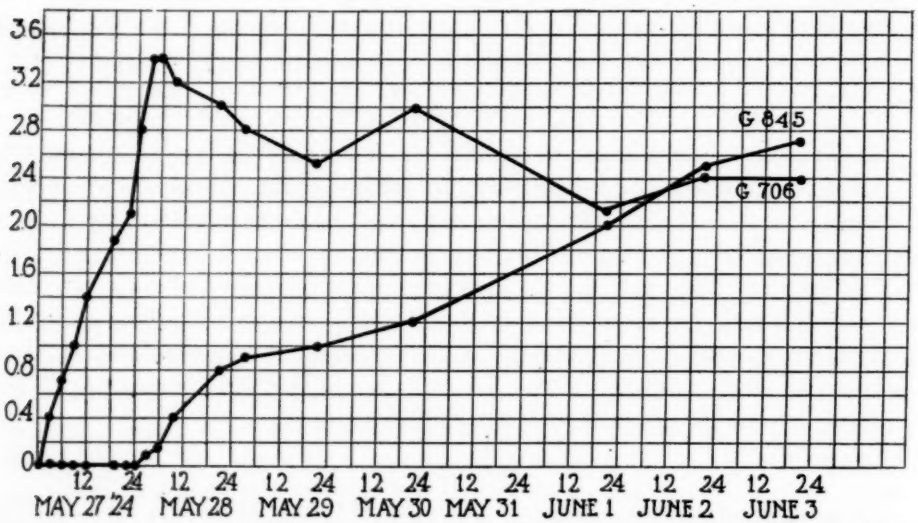

Fig. 1.—Curve showing the accumulation of bilirubin in the plasma of two dogs following obstruction of the common bile duct. At *0* both animals had their common bile duct sectioned and ligated. In Dog G845 the remainder of the biliary tract was not disturbed, while in Dog G706 the gall bladder was removed. The figures are given in van den Bergh units.

the plasma of the two series from three to thirty-six hours after operation gives a clear colorless plasma in the first, and a definitely yellow plasma in the second. From thirty-six to eighty-four hours after operation the plasma of the first series is a pale yellow, while that of the second is a deep yellow. The van den Bergh test on these plasmas demonstrates even more clearly the slow development of bilirubin in the first series, compared with the rapid development in the second series. The direct reaction is given by the van den Bergh test only after thirty-six to forty-eight hours in the first series, and within three to six hours in the second series. In all cases in which the indirect reaction was positive, the direct reaction was also positive, although it was somewhat delayed on the plasma containing small amounts of pigment.

DISCUSSION

The marked difference in the rate of the accumulation of bile pigment in the animals in which the common bile duct is obstructed, and in those with

similar obstruction and removal of the gall bladder illustrates strikingly the remarkable activity of the gall bladder. Inspection of the plasma or serum obtained from these animals any time from three to forty-eight hours after ligation of the common bile duct, shows clearly which animals are without gall bladders, and which have normally functioning gall bladders. This experiment could be utilized as a classroom demonstration to show that the gall bladder has a function. The same difference in time of appearance of jaundice is noted if the cystic duct is ligated, and the gall bladder allowed to remain intact. Moreover, if the gall bladder is severely injured, the same rapid accumulation of pigment follows ligation of the common duct. The same rapid appearance of pigment occurs after ligation of the common duct in animals that have been subjected to cholecystectomy several weeks previously, resulting in greatly dilated hepatic and common ducts. This fact eliminates, to a considerable degree, the mechanical factor which might otherwise be considered, that the elasticity of the gall bladder prevents the early accumulation of pigment when the gall bladder is present in obstructed animals.

SUMMARY

Following ligation of the common bile duct, signs of icterus, that is, bile pigment in the urine, plasma, sclerotics and mucous membranes, do not develop for from thirty-six to forty-eight hours after operation. If the gall bladder is removed when the common duct is ligated, the urine and plasma contain definite amounts of bile pigment within three to six hours after operation, and the sclerotics and mucous membranes are definitely yellow within twenty-four hours. The difference in the rate of accumulation of bile pigment is considered as evidence of the ability of the gall bladder to concentrate the bile secreted by the liver.

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THE EFFECT OF SPECIFIC CHOLECYSTITIS ON THE BILE-CONCENTRATING ACTIVITY OF THE GALL BLADDER*

By Jesse L. Bollman, M.D., Frank C. Mann, M.D., and Pierre DePage, M.D., Rochester, Minn.

IN another paper we have shown that the presence of the gall bladder is sufficient to prevent bile pigment from appearing in the blood or urine of a dog[3] for more than twenty-four hours following ligation of the common bile duct. If the gall bladder is removed, or the cystic duct ligated, at the time the common bile duct is ligated, bilirubin appears in the blood and urine within three hours after obstruction of the common bile duct. With this method for testing the bile-concentrating activity of the gall bladder, we studied the effect of a specific cholecystitis on this activity. One of us has called attention to the chemical means of producing a specific cholecystitis by the intravenous injection of sublethal amounts of chlorinated soda (Dakin's solution). In this manner an acutely inflamed gall bladder may be produced almost immediately following injection, with but slight coincident changes in other organs. The lesions in the gall bladder may persist for a number of weeks after all evidence of disturbance has disappeared from other organs. This observation has been confirmed, in experiments on the dog, by Peterman, and by Sweet and Reimann. Stenhouse reported several cases of cholecystitis in man which seem to be the result of inhalation of poison gas.

METHOD OF RESEARCH

Dogs were used in the experiments. Three or four days previous to operation, the animals were injected intravenously with 10 c.c. for each kilogram of body weight, of a solution of chlorinated soda (Dakin's solution). Double ligation of the common bile duct with section of the duct between the ligatures was performed under ether anesthesia with aseptic technic. The remaining portion of the biliary tract was not disturbed. The gall bladder was examined *in situ*, and the extent of the gross lesions recorded. Observations were subsequently made on the time of appearance and rate of accumulation of bile pigment in the plasma and urine. The animals were examined at necropsy to make certain that the ligated bile ducts had not ruptured. The van den Bergh method was employed for the detection of bilirubin in the plasma.

RESULTS

There was a surprising parallelism between the time of appearance of bile pigment in the plasma and the gross appearance of the gall bladder at the time of operation. The various animals developed bile pigment in the plasma and urine at widely different times, and the pigment accumulated in the blood

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at widely different rates in accordance with the variations in extent of the lesions in the gall bladder. The tests of some animals for bilirubin in the blood and urine were positive as early as three hours after ligation of the common bile duct, and the accumulation of bile pigment in the blood was rapid, so that the animals appeared jaundiced in less than twenty-four hours. A similar picture is obtained following ligation of the common bile duct and extirpation of the gall bladder. All of the animals that developed bile pigment in the blood and urine so rapidly following ligation had been observed to have a most severe inflammation of the gall bladder at the time of operation, the entire organ appearing to be involved.

In another group of animals the tests for bilirubin in the blood or urine were not positive until twenty-four to thirty-six hours after ligation of the

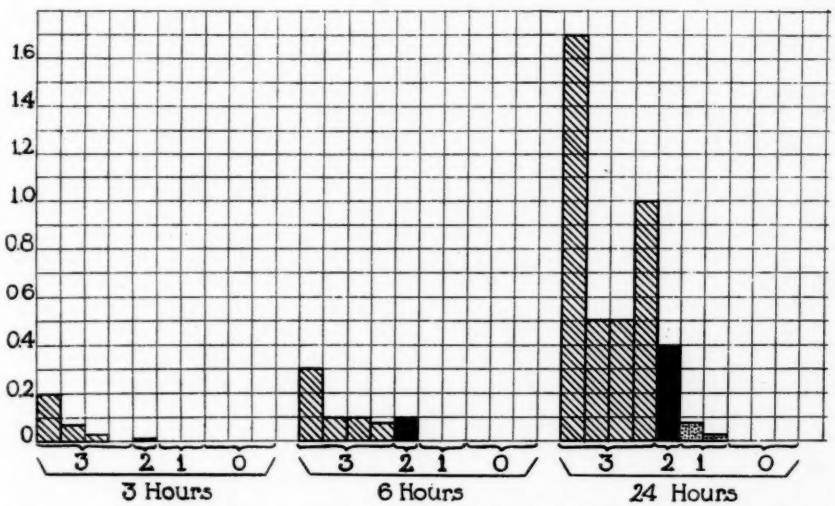

Fig. 1.—Chart showing the development of jaundice in relation to the severity of lesions of the gall bladder. Estimations of plasma bilirubin three, six and twenty-four hours after ligation of the common bile duct in animals with a normal appearing gall bladder, *0;* in animals with a very mild degree of cholecystitis, *1;* in animals with a moderate degree of cholecystitis, *2*; and in animals with a very severe cholecystitis, as judged by inspection at the time of operation, *3.*

common bile duct. This finding is similar to that following ligation of the common bile duct in an otherwise normal animal. In these animals, no lesion of the gall bladder was observed at the time of ligation of the common bile duct.

The remaining animals of this series developed bilirubin in the blood and urine at periods between the extremes mentioned. Animals with more severely inflamed gall bladders showed signs of jaundice earlier than those with milder and less extensive lesions (Figs. 1 and 2).

DISCUSSION

The activity of the gall bladder in concentrating the bile is clearly shown to be impaired by inflammatory lesions of the gall bladder. If these lesions are extensive, involving almost the entire organ, this activity is apparently

entirely lost. The functional significance of this activity of the gall bladder is by no means certain, but it is easily disturbed by the presence of acute lesions in the organ. It will be interesting to determine the permanent effect of an acute inflammatory process on this activity, and the influence of a chronic process, with a view to ascertaining whether or not the activity returns with the subsidence of the inflammatory process.

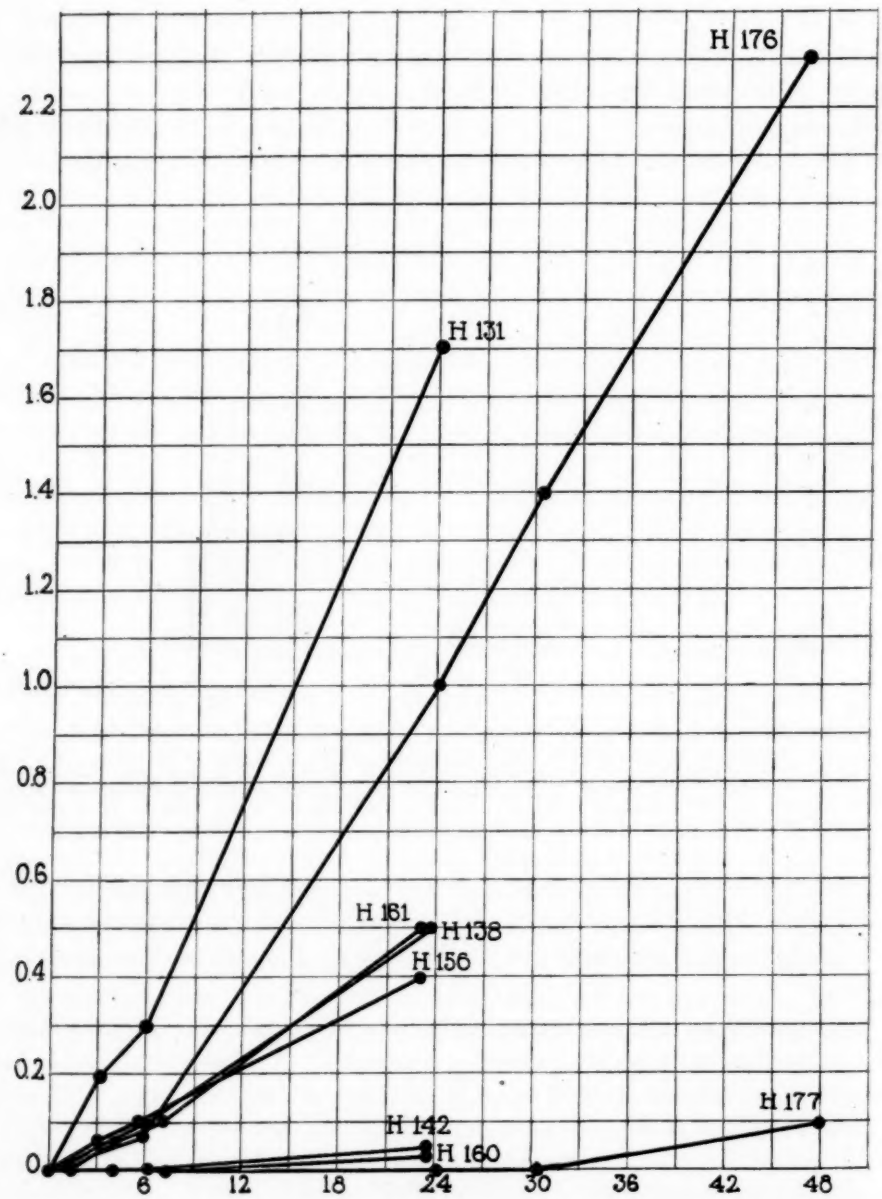

Fig. 2.—Curve showing the development of bilirubin in the plasma following ligation of the common bile duct. Dogs H131 and H176 had a severe cholecystitis involving the entire gall bladder; in Dogs H161, H138, and H156 about one-half of the gall bladder appeared to be involved; in Dogs H142 and H160 the gall bladder was but slightly involved; Dog H177 was a normal animal. The bilirubin estimations are in van den Bergh units.

SUMMARY

A specific cholecystitis has been produced in dogs by the intravenous injection of solutions of chlorinated soda. The time of appearance and rate of development of bile pigment in the blood and urine of these animals following ligation of the common bile duct depends on the degree of involvement of the gall bladder by this acute process. In animals in which the whole gall bladder seemed diseased, jaundice developed as rapidly as in animals in which the gall bladder was removed, and the common bile duct ligated similarly. Animals having milder disease of the gall bladder developed jaundice less rapidly, and animals with but slight or no apparent disease developed jaundice at the same rate as normal animals following ligation of the common bile duct. These facts clearly demonstrate that the function of the gall bladder of concentrating the bile is impaired, or may be totally lost, in the presence of an acute inflammatory process in the gall bladder.

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ON THE APPLICATION OF THE HENCH-ALDRICH UREA INDEX TO THE SPINAL CORD*

By George S. Graham and Sarah H. MacCarty

A RAPID method for the determination of the urea content of the saliva has recently been developed under Rountree's direction by Hench and Aldrich.[1] It is based on Friedlander's titrimetric method for the estimation of the urea content of the urine. It requires for its application only the simplest of apparatus and reagents, and yet appears capable of returning to the clinician information of considerable value. The method depends upon the fact that urea combines with mercury salts to form stable compounds. The amount of urea contained by a given solution will therefore be proportional to the amount of mercury bound by it. The test consists in adding from burette a 5 per cent solution of mercury bichloride to 5 c.c. of saliva and determining by an indicator solution of sodium carbonate the point at which the mercury-combining power of the salivary constituents is satisfied and free mercury ions remain in the solution. The amount of mercury solution calculated as necessary to saturate 100 c.c. of saliva is taken as the "Urea Index." Certain other substances are capable of binding mercury salts. Most important of these biologically are ammonium salts, uric acid, creatinine and amino acids. Hench and Aldrich found in practice that the mercury bound by all salivary bodies other than urea and ammonia nitrogen was, in normal individuals, 39 per cent of the total amount taken up; in patients with urea retention, 35 per cent. The test was found to have a dependability of about 90 per cent. That is, with normal values for the salivary urea index there was, in nearly 90 per cent of cases, no urea retention in the blood. With a salivary index above the normal, definite urea retention was proved in 91 per cent of cases. In urea retention there was a close parallelism between the figures for the salivary index and those of the blood urea as determined by the urease method.

During the past winter the method was taken up in this laboratory. We were soon convinced that the claims made for its simplicity and usefulness were borne out in practice. The question then arose as to the applicability of the method to the determination of the urea content of the spinal fluid. The body membranes are highly permeable to urea and the substance is rather evenly distributed throughout the tissues and fluids. On the other hand, the choroid plexus holds back from the spinal fluid a considerable percentage of other substances capable of combining with mercury. Theoretically the spinal fluid should show a more highly specific urea-combining value than does the saliva. Myers and Fine[2] found that while the urea

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content of the spinal fluid was 88 per cent that of the blood, the creatinine was only 46 per cent, and the uric acid but 5 per cent. There is, however, some difference of opinion as to the actual relationship existing between the urea content of the spinal fluid and that of the blood. Woods[3] gives the two values as approximately the same. Myers and Fine[2] found considerable variation in different cases. In four instances from a series of fourteen cases, the spinal fluid urea was from 100 per cent to 121 per cent that of the blood; in six cases it was from 90 per cent to 95 per cent; in three cases, from 81 per cent to 87 per cent; and in one case, 69 per cent. Cullen and Ellis[4] made a series of determinations on thirty-two patients, all of whom appear to have been suffering from tabes. In 63 per cent of their cases the difference between the urea content of blood and of spinal fluid was less than 2 milligrams per 100 c.c. The greatest difference found was 11 milligrams. The urea values varied from 20 to 42 and from 22 to 46 milligrams per 100 c.c. for serum and spinal fluid respectively.

From these and similar studies it would appear that in most cases the spinal fluid contains urea in a concentration approximating that of the blood. A method adapted to measuring this urea simply and rapidly should have considerable clinical value. Routine determinations in diagnostic lumbar puncture fluids might give incidental information as to urea retention and might occasionally point the way to diagnosis in otherwise obscure cases of uremic coma. With such considerations in mind, the attempt has been made to find out whether the Hench-Aldrich test may be expected to give results for the spinal fluid as dependable as those claimed for it in the saliva. In a small series of cases the mercury has been determined in parallel with urease determinations on spinal fluid and blood. In a larger series it was not practicable to run urease determinations. Here the mercury index alone was obtained. These simple figures are not, however, without some interest. In a few cases the test was applied to spinal fluids obtained at autopsy. The technic used throughout was the same as that of the original method, the only change consisting in the substitution of 5 c.c. of spinal fluid for 5 c.c. of saliva. The controlled urea index findings are given in the following table:

TABLE I

CASE	SEX	AGE	DIAGNOSIS	MERCURY UREA INDEX SP. FLUID	UREA N BY UREASE, SP. FLUID	UREA N BY UREASE, BLOOD
1	F	30	Myocarditis	28	8.47	10.7
2	M	35	Syphilis	36	–	13.9
3	M	28	Syphilis	45	18.75	20.4
4	M	62	Lymphatic leucemia	48	15.7	18.48
5	M	50	Brain tumor (?); Lues (?).	50	16.7	18.48
6	M	39	Fracture of skull	64	19.05	–
7	M	52	Cardiorenal disease	128	46.8	54.35
8	M	30	Sickle cell anemia; streptococcic pneumonia	153	58.35	–
9	M	60	Myocarditis; bronchopneumonia	260	119.0	128.0
10	M	54	Prostatic hypertrophy	258	73.65	78.6

The results in these few cases seem sufficiently clear to warrant further trial of the method. For the first eight cases there is approximate agree-

ment between the values obtained by the mercury titration and by the urease method. As between the last two cases, a difference of 45 milligrams in the urease levels is associated with a difference of only 2 in the urea indices. It is probable that the discrepancy here is due to a distortion of the index in Case 10. During titration a heavy white curdy precipitate appeared. Smaller amounts of such a precipitate have been seen not uncommonly. It has been particularly noticeable in postmortem spinal fluids. When present in abundance the substance or substances producing it may act as a buffer or as a direct binder of the mercury and so defer the reaction between mercury and urea. It is possible that a perfected technic may serve to eliminate this possible source of error.

Concerning the fundamental question as to the relative concentration of urea in the spinal fluid and in the blood, our average results agree with those of Myers and Fine. We have not encountered so wide a range of variation as appeared in their series. Cullen and Ellis, Woods and earlier writers appear to have obtained percentages more constant than our own. There is evident need here for further study, particularly with a view to determining whether the ratio may vary in different types of disease. The question has, of course, a practical bearing for the present study, since upon its answer must depend much of the clinical value of any method such as that now proposed.

In a series of twenty unselected cases, the urea index was taken as a single factor unsupported by more exact chemical determinations. The lowest value, 34, was obtained in a case of tetanus in a nine-year-old child. Three patients gave a value of 36 and six gave values of 36 to 50. In two of the latter group there was some urinary evidence of renal disturbance. In one, a forty-five-year-old man, under intraspinal treatment for cerebrospinal syphilis, three tests covering a period of one month gave indices of 40, 50 and 45. The urine showed a trace of albumin but no casts. In the other, a man of twenty-three years suffering from acute Staphylococcus meningitis, an index of 48 was accompanied by a trace of albumin in the urine. In general it may be said that urinary or other evidence of nephritis begins to appear in the series at a urea index level of about 50. A case of chronic suppurative mastoiditis in a man of twenty-five years with an index of 50 showed urinary albumin and a few hyaline casts. On the other hand, urinary findings were negative in a case of tabes dorsalis with an index of 52.5 and again in a case of cerebrospinal syphilis in which five tests taken over a period of seven weeks gave indices of 54, 54, 68, 50 and 50. An index of 55 in an eleven-months-old infant that died twenty-four hours later of streptococcic meningitis could not be checked. In a nine-months-old infant with "breast milk poisoning," spinal fluid obtained one hour before death gave an index of 62.5 and the urine showed a trace of albumin, many hyaline casts and a 4-plus acetone reaction. An index of 63 in a seventy-six-year-old man with prostatic hypertrophy was accompanied by a urinary specific gravity of 1005, a faint trace of albumin, and occasional hyaline casts. Three cases gave indices of 100 to 116.6. One of these was a child of four years with acute suppurative otitis media and bronchopneumonia; the urea index was 116.6.

The urine showed a specific gravity of 1021; a trace of albumin; pus content rated as 2-plus; there were many hyaline, granular and cellular casts. The other two cases of high index figures were adult males with obvious renal disease.

Spinal fluid was obtained at autopsy in seven cases. The mercury index here was usually high and had obviously no relationship with antemortem urea retention. The lowest value, 46, was found in the fluid from a child of four years who died of hydrocephalus consequent upon a large solitary tubercle of the cerebellum. The fluid was obtained twelve hours after death. A value of 85 was obtained twenty-four hours after death in a man fifty years of age who died three hours after fracture of the skull. The kidneys showed only microscopic sclerosis. Four cases had indices of 112 to 204 without anatomic lesions sufficient to explain a urea retention. The highest value, 737.5, was obtained in a two-months-old infant that died of acute purulent meningitis. Centrifuged fluid was used but there was long-persisting formation of a curdy precipitate as the mercury was added.

In three cases we had the opportunity of examining the fluid both before and after death. In one, Case 9 of Table I, the index was 260, ten hours before death and 428, eight hours after death. In a second case of hydrocephalus, an index of 40 was obtained two days before death. Seven hours after death, fluid from the lateral ventricle gave an index of 78. In a case of tuberculous meningitis an index of 64 on the second day preceding death had changed to one of 208 eighteen hours after death. Such findings are in agreement with those of Myers,[5] who showed that no value could attach to postmortem study of the spinal fluid. It is probable that waste and disintegration products begin to accumulate during the agonal period and that they mount rapidly immediately after death.

SUMMARY AND CONCLUSIONS

An attempt has been made to apply the Hench-Aldrich test for salivary urea to the determination of the urea content of the spinal fluid. The procedure is simple and a determination can be made in a few minutes. It is not an exact chemical method comparable to the well-established procedures commonly employed and it is still questionable how far it may be expected to agree with these. The work is being continued with the view to answering this question. The indications are, however, that it will prove sufficiently delicate and reliable to detect any distinct urea retention in the body fluids and that it may be expected to give an approximate determination as to the degree of this retention. Its possible value as a useful sign in obscure cases of uremic coma is evident. The normal urea index appears to range from about 30 to about 50.

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THE READING OF COMPLEMENT-FIXATION TESTS BY THE CITRON SCALE AS COMPARED WITH A METHOD USING A COLOR SCALE*

By Ruth Gilbert, M.D., A.M., Margaret F. Kelley, A.B., M.A., and Anna C. Moore, A.B.

THE scale of reading complement-fixation tests quite generally adhered to in this country is an adaptation of the Citron scale,[1] in which the fixation obtained in the two amounts of serum tested is recorded as follows:

1. Fixation complete with both 0.02 c.c. and 0.01 c.c. of serum = 4+
2. Fixation complete with 0.02 c.c. and nearly complete with 0.01 c.c. = 3+
3. Fixation complete with 0.02 c.c. and partial with 0.01 c.c. = 2+
4. Fixation nearly complete with 0.02 c.c., partial or slight with 0.01 c.c. = +
5. Fixation partial or slight with 0.02 c.c., partial or slight with 0.01 c.c. = ±
6. Complete hemolysis in both tubes = no complement fixation obtained.

There are two objections to this scheme:

1. This scale of values has been found misleading because of the wide range of + from nearly complete fixation to only partial fixation, so that a reaction which may be relatively strong is reported as only + and is frequently interpreted as having no clinical significance.
2. The scheme is not uniformly applied by different workers because of the variations in the individual's conception of the terms, nearly complete, partial or slight fixation.

It, therefore, seemed desirable to adopt a scale in which a more definite valuation would be given to sera giving nearly complete fixation in the larger amount, and to establish this scale upon standards which could be more uniformly applied. With this end in view, the Citron method of reading was compared with a method in which the exact degree of inhibition of hemolysis was estimated by comparison with color standards containing varying percentages of hemolyzed and nonhemolyzed cells.

The use of such a color scale for reading complement-fixation tests was advocated as early as 1910 by Thomsen and Boas,[2] working in Madsen's laboratory. Verne,[3] in France, devised an ingenious artificial scale for this purpose. Bergeron and Normand[4] found, however, that the colors in the Verne scale did not exactly correspond to the color of hemolyzed cells, so they introduced a natural scale using varying percentages of hemolyzed cells. In this country, Mahr[5] has reported on the use of an artificial color scale, and Terry[6] on a natural one made up with varying proportions of cells and hemoglobin solution. Ivy[7] has recommended the use of the Duboscq colorimeter for estimating the degree of hemolysis, which is an accurate but complicated method. Kolmer[8] also recommends the use of a color scale and points out that it should contain, in addition to the varying proportions of sheep's corpuscles and

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hemoglobin solution, antigen and complement in the same amount as used in the test.

Technic Used in the Complement-Fixation Tests on which Comparative Readings were Made.—The total volume of the test was 0.5 c.c., i.e., one-tenth that of the original Wassermann test. All reagents were diluted in accordance with preliminary titrations so that a quantity of 0.1 c.c. might be pipetted. The complement was titrated daily with five per cent sheep cells sensitized with two units of amboceptor and two units of complement were used in the test. Patient's serum was inactivated for one-half hour at 54°-56° C. It was tested in two amounts, 0.02 c.c. and 0.01 c.c., which represent quantities of 0.2 c.c. and 0.1 c.c. in terms of the original Wassermann test. Each specimen was tested with two antigens, a crude alcoholic extract of beef heart and an alcoholic extract of beef heart reinforced with 0.4 per cent cholesterin. The antigens used were not even slightly anticomplementary in four times the amount used in the test and contained from two to four times the quantity of antigen required to give complete fixation with known "four plus" syphilitic serum. The tests with both antigens were fixed for four hours at 3°-6° C. Five per cent sheep cells sensitized with two units of amboceptor were then added and the tests placed in the water bath at 37° C. until controls, containing serum alone and antigen alone, showed complete hemolysis. Readings were then made by the Citron method and by comparison with color standards.

Preparation and Use of Color Scale.—Ten c.c. of the five per cent sheep cell suspension were centrifugalized at high speed for twenty minutes. The supernatant salt solution was then drawn off and the cells laked by adding sufficient distilled water to bring the volume up to 9.0 c.c. Then 1.0 c.c. of 8.5 per cent salt soltion was added to make it isotonic and the solution was mixed thoroughly. A small amount of this 100 per cent hemoglobin solution was diluted 1-10. Twelve standards were prepared as indicated in the following diagram:

DIAGRAM OF COLOR STANDARDS

DEGREE OF INHIBITION OF HEMOLYSIS	100%	98%	95%	90%	85%	80%	75%	60%	50%	40%	25%	10%
Hemoglobin solution diluted 1-10 (c.c.)	0	0.02	0.05	0	0	0	0	0	0	0	0	0
Hemoglobin solution undiluted (c.c.)	0	0	0	0.01	0.015	0.02	0.025	0.04	0.05	0.06	0.075	0.09
5% sheep cell suspension (c.c.)	0.1	0.098	0.095	0.09	0.085	0.08	0.075	0.06	0.05	0.04	0.025	0.01
Two unit complement (c.c.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Antigen (c.c.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
0.85% salt solution (c.c.)	0.2	0.182	0.155	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

The contents of these standard tubes were equal in volume to the test tubes of patient's serum and contained the same ingredients, with the exception of patient's serum and the substitution of 0.85 per cent salt solution for diluted amboceptor. The tubes containing the standards ranging from 100 per cent through 50 per cent inhibiton were centrifugalized. Those showing 40 per cent or less inhibition were left with the cells in suspension. Tests showing 40 per cent or less inhibition of hemolysis were compared with the standards containing cells in suspension. Those showing more than 40 per cent inhibition were centrifugalized and the colors of the supernatant liquids compared with those of the supernatant liquids of the centrifugalized standards. These standards were prepared each day. In every case in which the

patient's serum was tinged with hemoglobin, a control was made containing the amount of patient's serum used in the test, 0.2 c.c. of sensitized cells and 0.85 per cent salt solution, to bring the volume up to 0.5 c.c. This was centrifugalized and the color of the supernatant liquid compared with that of the color standards. The per cent hemolysis to which this control corresponded was subtracted from that shown by the test itself, the difference being the actual per cent hemolysis shown by the serum in question.

Comparison of Results with Two Methods of Reading.—In order to compare the results of these two methods of reading, it was necessary to translate the amounts of inhibition of hemolysis observed by the use of color standards into the degrees of definite and partial fixation represented by the terms ±, +, 2+, 3+ and 4+. The scale tentatively adopted, based on the 0.02 c.c. amount of serum, was that sera showing from 10 to 20 per cent inhibition of hemolysis should be considered ±; those showing from 25 to 45 per cent inhibition +; those from 50 to 70 per cent inhibition 2+; those from 75 to 95 per cent inhibition 3+; and those showing 98 to 100 per cent inhibition 4+. This seemed the most logical scale, and the clinical data of the borderline cases indicated that it was safe to place the lower limit of definite fixation at 50 per cent inhibition of hemolysis.*

TABLE I

COMPARATIVE RESULTS OF 2051 COMPLEMENT-FIXATION TESTS WITH THE PLAIN ALCOHOLIC ANTIGEN READ BY THE CITRON METHOD AND BY A METHOD USING A COLOR SCALE

CITRON METHOD	PERCENTAGE INHIBITION OF HEMOLYSIS IN 0.02 C.C. SERUM					
	TOTAL	10-20 (±)	25-45 (+)	50-70 (2+)	75-95 (3+)	98-100 (4+)
Total	2051	177	178	119	424	1153
±	376	177	159	40	0	0
+	245	0	19	78	134	14
2+	21	0	0	1	17	3
3+	145	0	0	0	115	30
4+	1264	0	0	0	158	1106

TABLE II

CLINICAL HISTORIES OF THE 266 CASES IN WHICH COMPLEMENT-FIXATION TESTS MADE WITH PLAIN ALCOHOLIC ANTIGEN WERE READ AS + OR ± BY THE CITRON METHOD AND 4+, 3+ OR 2+ BY THE USE OF A COLOR SCALE

NUMBER OF SPECIMENS	CLINICAL HISTORY
266	Total Cases
178	Treated cases of syphilis
27	Untreated cases diagnosed as syphilis
26	Untreated cases with history or symptoms suggestive of syphilis
2	Cases with immediate members of family syphilitic
5	Cases without definite history but stated to have had a previous blood test giving definite complement fixation
21	Cases without definite history but giving definite complement fixation with the cholesterinized antigen by both methods of reading
7	Cases with data inadequate for classification
1	Acquired syphilis? Weakness, 5 years
1	Arthritis
1	Headache 4 years
1	Auditory neuritis
3	No history given

*It was found in a small series of comparative readings that, in grouping the results according to this scale, it was sufficient to use only the limiting standards of 25 per cent, 50 per cent, 75 per cent and 95 per cent inhibition of hemolysis.

TABLE III

COMPARATIVE RESULTS OF 2331 COMPLEMENT-FIXATION TESTS WITH CHOLESTERINIZED ANTIGEN READ BY THE CITRON METHOD AND BY A METHOD USING A COLOR SCALE

CITRON METHOD	PERCENTAGE INHIBITION OF HEMOLYSIS IN 0.02 C.C. SERUM					
	TOTAL	10-20 (±)	25-45 (+)	50-70 (2+)	75-95 (3+)	98-100 (4+)
Total	2331	72	154	109	351	1645
±	242	72	138	32	0	0
+	320	0	16	77	199	28
2+	37	0	0	0	20	17
3+	131	0	0	0	67	64
4+	1601	0	0	0	65	1536

Two thousand and fifty-one comparative readings were made with the plain alcoholic antigen. The comparative results of these tests by the two methods of readings are shown in Table I. Inasmuch as approximately 12.5 per cent of the tests read 4+ by the Citron method actually showed from 5 to 20 per cent hemolysis, it is evident that this degree of hemolysis is not always recognized with the cells in suspension. There is also an error in the other direction, as approximately 6 per cent of the tests read + by the Citron method showed no hemolysis in the 0.02 c.c. amount of serum. Though there would have been some redistribution of the tests which read 4+, 3+ or 2+ by the Citron method on the basis of the color standard readings, they would all have been reported as giving definite fixation of complement by either method of reading. However, of the 621 tests read + or ± by the Citron method, 266, or slightly over 40 per cent, were read 2+, 3+ or 4+ with the use of a color standard. The clinical histories of these 266 cases are given in Table II.

Two thousand three hundred and thirty-one comparative readings were made with the cholesterinized antigen. The comparative results of these tests by the two methods of reading are given in Table III. These results likewise indicate the errors in reading tests with cells in suspension, since in approximately 4 per cent of the cases read 4+ by the Citron method, there was actually from 5 to 20 per cent hemolysis, and almost 9 per cent of those which read + showed no hemolysis in the larger amount when read with a color standard. Of the 562 tests which read + or ± by the Citron method, 336 or approximately 60 per cent were read 2+, 3+, or 4+ with the use of a color standard. The clinical histories of these 336 cases are given in Table IV.

An analysis of the histories given in Tables II and IV indicates that reading complement-fixation tests by the use of color standards, with the range of 50 to 100 per cent inhibition for definite fixation, gives results more in conformity with the clinical findings than those obtained by the use of the Citron scale.

Summary and Conclusions.—The readings of tests after centrifugalization by recording the percentage inhibition of hemolysis as compared with a color standard brings out the fact that the Citron method of reading with the cells in suspension does not, in many instances, give an exact estimate of the amount of fixation that has occurred. Comparative readings have shown that from 5 to 20 per cent hemolysis is frequently not recognized with the cells in suspension, so that sera showing less than 20 per cent hemolysis in both

TABLE IV

CLINICAL HISTORIES OF THE 336 CASES IN WHICH COMPLEMENT-FIXATION TESTS MADE WITH THE CHOLESTERINIZED ANTIGEN WERE READ AS + OR ± BY THE CITRON METHOD AND 4+, 3+ OR 2+ BY THE USE OF A COLOR SCALE

NUMBER OF SPECIMENS	CLINICAL HISTORY
336	Total Cases
238	Treated cases of syphilis
31	Untreated cases diagnosed as syphilis
21	Untreated cases with history or symptoms suggestive of syphilis
2	Cases with immediate members of family syphilitic
5	Cases without definite history but stated to have had a previous blood test giving definite complement fixation
39	Cases with data inadequate for classification
1	Trifacial neuralgia
1	Acquired syphilis? Weakness, 5 years
1	Dizziness, two weeks
1	Weakness and dizziness
1	Pneumonia, two weeks
1	Rash on limbs
1	Endarteritis of leg
1	Indolent ulcer foot and hands, 1 month
1	Delayed healing of wound
1	Paralysis agitans
1	Headache, six weeks
1	Headache and nervousness, not considered syphilitic by the attending physician
1	Psychosis, case from insane hospital
1	Prostitute, involutional melancholia, no physical signs of syphilis
1	Manic depressive insanity and history of three miscarriages; attending physician considered it unlikely that lues entered into etiology
1	Negress, syphilis denied
1	Inmate of state prison, venereal disease denied
3	Inmates of the state prison, syphilis denied, gonorrhea admitted
19	No history given

amounts are read 4+, but with 20 per cent or less hemolysis in the 0.02 c.c. amount and 30 per cent or more in the 0.01 c.c. amount are usually read +, making the apparent difference in the reading greater than the actual difference in degree of fixation. The Citron method of reading is misleading because the reports on two specimens which give nearly complete fixation in the larger quantity of serum, may vary between 4+ and +, whereas careful comparison with a color standard, using the scale of reading proposed, would result in reports on all such specimens of definite complement fixation with a quantitative difference based on the percentage inhibition of hemolysis observed. This would tend to make more uniform the results reported on successive specimens from the same patient and the results reported by workers in different laboratories. The scale adopted results in an increase in definite reactions and a decrease in the partial reactions which are so misleading and confusing to the clinician. At the same time the limits have been so placed as to avoid giving too great significance to weak or slight fixation.

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THE CHLORIDE CONTENT OF THE BLOOD IN PERNICIOUS ANEMIA*

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CERTAIN facts suggest a possible relation of chloride metabolism to pernicious anemia. Free hydrochloric acid is practically constantly absent from the gastric juice in this disease. The presence of free acid makes the diagnosis of pernicious anemia extremely questionable. Levine and Ladd[1] found an achlorhydria in 104 of 105 patients. In Cabot's series[2] 78 of 79 patients showed an absence of free hydrochloric acid. I have reported[3] 50 cases in 47 of which a gastric analysis was done and free acid found absent in every instance. It is highly probable that the occasionally reported finding of free hydrochloric acid represents an error in technic or in diagnosis.

Pernicious anemia is a hemolytic disease of toxic origin. The gastrointestinal tract is probably the site of origin of the toxin. Some observers hold that the absence of free hydrochloric acid is the immediate cause for the development of the toxin. Others with equally good reasons think that the disappearance of free acid is a result and not a cause. According to the latter view, the achlorhydria, the blood destruction, the bone marrow changes, the glossitis and the characteristic nervous system lesions are all manifestations of the action of an unknown toxin.

The toxin responsible for the toxemia of high intestinal obstruction is possibly closely related to the toxin of pernicious anemia. Numerous cases have been reported in which a blood picture indistinguishable from idiopathic pernicious anemia has occurred with either a benign or a malignant stenosis of the small intestine.[4] Such seemingly represent cause and effect rather than coincident diseases. The fundamental and characteristic feature of the chemical pathology of high intestinal obstruction is a disturbance of chloride metabolism.[5] The well-recognized occurrence of a blood picture resembling pernicious anemia in certain cases of carcinoma of the stomach is also of interest in this connection. It is highly probable that in such cases there is a toxin elaborated identical with or similar to that responsible for idiopathic pernicious anemia. Emerson[6] found that in carcinoma of the stomach there are substances in the gastric contents which tend to

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neutralize hydrochloric acid. Since these substances are destroyed by heat they are most probably of the nature of ferments.

A final point in the similarity of the toxemia of pernicious anemia and intestinal obstruction is the occurrence of melanuria in both conditions. Melanuria, in the absence of a melanotic tumor, as Haden and Orr[7] have shown, is indicative of specific type of protein destruction occurring only after the depletion of the blood chlorides between a certain level.

To determine if possible whether a disturbance in chloride metabolism can be found in pernicious anemia, I have made chloride determinations on the blood of all patients with pernicious anemia seen during the past two years. For comparison with these findings, determinations have also been made on numerous cases of secondary anemia.

Other workers have made observations on the chlorides in anemia. Host[8] determined the blood chlorides in several cases and found the plasma content about that of normal blood while the whole blood content tended to be higher. Buckman and Edwards[9] found no depression of chloride content of the red blood cells peculiar to pernicious anemia. They obtained low values for chlorides in all types of anemia with very low cell volumes. Essen and Porges[10] found that the sodium chloride of the blood in anemia lay at the upper level of normal and tended to fall as the anemia improved. Christian[11] has investigated the renal response to the nephritic test meal in pernicious anemia. He found a very low excretion of nitrogen and salt and concludes that this is due to an impairment of kidney function. In cases in which successive tests were made with an improvement in the anemia, there was a marked rise in salt excretion usually out of proportion to the increase in nitrogen. It seems quite possible that the changes in salt excretion observed may represent a disturbance in chloride metabolism rather than in kidney function.

In the cases reported here the chloride determinations were made as part of a complete blood study. Since chlorides are usually much higher in plasma than in corpuscles it is necessary to know the relative cell and plasma volumes. The cell volume in each case was determined by centrifuging 10 c.c. of blood with 2 c.c. of 1.6 per cent (isotonic) solution of sodium oxalate. The volume index was calculated in each instance and reported elsewhere.[5] The chloride determinations were made on the tungstic acid filtrate by iodometric titration after the method suggested by Gettler.[12]

The results in thirty consecutive cases of pernicious anemia are tabulated in Table I. The highest chloride value is 620 mg. per 100 c.c. and the lowest 320 mg. The cases in which there is a low cell volume have higher whole blood chlorides than those in which the cell volume appears normal. The plasma chlorides are all within normal limits. The constancy of the plasma chlorides explains the higher values in bloods with low cell volumes since the chloride content of plasma is usually about 100 mg. per 100 c.c. higher than that of whole blood.

The total blood volume in anemia is little different from normal so there is actually a larger amount of circulating chloride per kilogram of body

weight in individuals with low cell volumes than in normal persons. There is no demonstrable relation of the volume index to the chloride content. In four cases of pernicious anemia (Nos. 22, 23, 28, 30) the whole blood chlorides are below the accepted lower limit of normal.

For comparison with the results in pernicious anemia I have tabulated in Table II in a similar manner the findings in twenty cases of secondary anemia. The results here are in general the same as in pernicious anemia. With low cell volumes the whole blood chlorides are higher than normal while the plasma content is within normal limits. Where the cell volume deviates little from normal, the chlorides show little change.

TABLE I

CHLORIDE CONTENT OF THE BLOOD IN PERNICIOUS ANEMIA

CASE NO.	RED BLOOD CELLS IN MILLIONS PER C.MM.	VOLUME OF RED BLOOD CELLS		VOLUME INDEX	CHLORIDES AS NaCl (MG. PER 100 C.C.)		FREE HCL IN GASTRIC JUICE
		PER CENT OF WHOLE BLOOD	PER CENT OF NORMAL		PLASMA	WHOLE BLOOD	
1	0.39	5	10	1.34		550	
2	0.59	8	17	1.42		600	0
	3.21	35	73	1.14		500	0
3	0.60	7	15	1.25	570	530	0
4	0.84	13	27	1.61	640	540	0
5	0.86	12	25	1.46	580	570	0
6	0.87	10	21	1.21		620	0
7	0.98	13.5	28	1.27	560	550	0
8	0.98	13.5	28	1.43		620	0
	0.98	14	29	1.45		520	
9	0.99	13.5	28	1.38		530	0
10	0.39	14	29	1.46		580	?
11	1.04	15.5	32	1.52	580	520	0
12	1.18	15	31	1.32	520	510	0
	0.97	13	27	1.38	570	530	
	2.67	31	65	1.23	575	510	
	4.60	44	95	1.03		445	
13	1.27	20	42	1.27		530	?
14	1.33	17	35	1.32	550	520	0
15	1.35	22	46	1.70	630	495	0
16	1.39	18	37	1.32		520	0
17	1.39	21	44	1.56	560	470	0
18	1.40	20	42	1.50		435	0
19	1.48	22	46	1.53	580	520	0
20	1.52	23	49	1.61		490	0
21	1.87	27	56	1.50	540	480	?
22	1.87	24.5	51	1.36	580	550	
	2.43	29	60	1.22		320	
	3.52					330	
	3.07	39	81	1.01		370	
	3.14	29	60	0.95		405	
23	2.18	27	57	1.31		400	0
	1.63	21	44	1.33	565	500	
	2.42			1.69	580	490	
24	2.23	36	75	1.69	570	480	0
25	2.41	37	76	1.57		420	0
	3.21	32	86	1.35	570	480	0
26	2.60	36	75	1.44	585	500	0
27	2.70	39	80	1.48	625	465	0
28	2.88	41	85	1.46		370	0
29	3.16	44	92	1.46		440	0
30	3.44	36	75	1.09		410	0

TABLE II

CHLORIDE CONTENT OF THE BLOOD IN SECONDARY ANEMIA

CASE NO.	RED BLOOD CELLS IN MILLIONS PER C.MM.	VOLUME OF RED BLOOD CELLS		VOLUME INDEX	CHLORIDE AS NaCl (MG. PER 100 C.C.)		DIAGNOSIS
		PER CENT OF WHOLE BLOOD	PER CENT OF NORMAL		PLASMA	WHOLE BLOOD	
1	1.03	9	19	0.91		560	Sepsis
2	1.69	15	31	0.91		540	Carcinoma of uterus
3	1.97	19	38.5	0.97	550	510	Malnutrition
4	2.01	20	42	1.00		520	Chr. nephritis
5	2.10	18	37	0.88		470	
6	2.16	22	45	1.04		540	Undetermined
7	2.18	21	43	1.00		490	Exhaust gas poisoning
8	2.26	22	46	1.00		530	Gastric hemorrhage
9	2.26	23	47	0.92		620	Banti's disease
	4.22	41	85	1.00		520	
10	2.95	27	56	1.20		470	Uterine hemorrhage
11	3.30	38	79	1.20		450	Hemolytic jaundice
12	3.40	28	58	0.87	570	520	Oral sepsis
	4.18	34	71	0.86	550	500	
13	3.42	31	65	0.96		490	Chr. cholecystitis
14	3.52	34	71	1.00		540	Pyelonephritis
15	3.56	36	75	1.05		470	Syphilis
16	3.69	29	61.5	0.83		470	Oral sepsis
	4.00	30	62.5	0.78		510	
	3.59	28	58	0.81	560	510	
17	3.78	37	77	1.02		470	Undetermined
	4.32	42	86	1.00		450	
18	3.78	32	68	0.90		470	Oral sepsis
19	3.79	36	75	0.99		466	Undetermined
20	4.65	29	62	0.67	610	570	Syphilis

These figures show that there is no constant variation in blood chlorides typical of anemia. It is still possible, however, that there may be a disturbance of chloride metabolism which is not reflected in the chloride level of the blood.

For comparison with the chloride content of the blood, I have noted in Table I, also the results of tests for free HCl acid in the gastric juice. None were present in any instance. There is no evident relation of the chloride level to the hydrochloric acid.

SUMMARY

Chloride determinations on thirty cases of pernicious anemia and twenty cases of secondary anemia are reported.

The whole blood chlorides are usually higher in blood with a low cell volume in both primary and secondary anemia.

The plasma chlorides remain about normal regardless of the cell volume.

A few cases of pernicious anemia show a low chloride content. No patients with secondary anemia had low chlorides.

There is no relation of the chloride level of the blood to the free hydrochloric acid of the gastric juice or to the volume index of the corpuscles.

The total store of chlorides in patients with very low cell volumes is higher than normal since the blood volume shows little variation from normal and the plasma chloride content is higher than whole blood.

These results give no positive evidence of a disturbance of chloride metabolism in pernicious anemia.

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LABORATORY METHODS

THE PAUL TEST IN THE DIAGNOSIS OF SMALLPOX*

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In a paper published about a year ago, the writers drew attention to the value of the so-called Paul method in the diagnosis of smallpox.[4] Since then, the work has been continued, and it is the purpose of the present paper to emphasize the principal conclusion which was reached at that time, that, histologically controlled, the method is reliable and deserves general application in all doubtful cases. We would point out particularly that the material used in the examination, viz., the dried exudate from vesicles and pustules, retains its infectivity for some time and can hence be transported to the laboratory from long distances. We have examined specimens sent, not only from neighboring states, but from far away states like Colorado, and even from distant countries like China, and found that the material had not suffered in transit in the least.

Our studies during the past winter have further shown that, as Gins[2] pointed out, the smallpox virus may at times be demonstrable in the nasal secretion when, on the basis of the usual criteria of recovery, the individual would be ready for discharge from quarantine. It is accordingly suggested that a careful rhinoscopic examination be made in every case before release, and that this be supplemented by an examination of the swabbings, with the same method, if there is the least suggestion of an ulcerative lesion.

TECHNIC

Principle of the Method.—In principle, the method is based upon the demonstration of macroscopically visible lesions in the inoculated rabbit cornea, in the sublimate alcohol bath, and the appearance of the affected areas in sections. The latter phase of the examination is unnecessary if, as is the rule, the macroscopic examination gives a clear-cut picture. In our own work, however, the histologic control was carried out in every instance.

The technic, as here described, is essentially that of Paul,[3] with certain minor modifications which suggested themselves in the course of our work.

Preparation of the Material for Inoculation.—A few vesicles or pustules are cleansed with alcohol, wiped dry with sterile cotton and opened with a sterile needle or a small lancet. The exuding vesicular or pustular material is mounted on clean slides in the form of *thick* drops. These are allowed to

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become air-dry and the specimens may then be wrapped in paper and sent to the laboratory in a suitable container. If the pustular stage has passed, a number of scales should be lifted from their beds and sent as such. Care should be taken, however, to secure only round, brown scales with a smooth surface. Yellow irregular crusts with fissured surfaces are usually devoid of virus. Serum from papules is not suitable for examination.

Inoculation of the Rabbit Cornea.—Both eyes of a rabbit are anesthetized with a few drops of a 5 per cent solution of cocaine hydrochloride, and then *delicately* scarified with a fine needle, making four or five horizontal and as many vertical scratches, about 1 mm. apart. A loopful of as heavy an emulsion in saline as can be prepared from the material submitted for examination, is then gently rubbed over the scarified surface of one eye. The other cornea serves for purposes of control. The animal is kept by itself in a cage, which is sterilized after use.

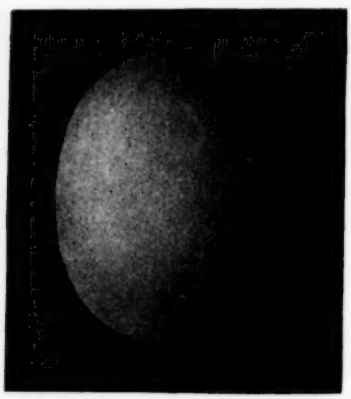
Examination of the Cornea in vivo.—This should be done with a hand lense or a binocular loupe, using a good lateral illumination. Paul makes his final examination forty-eight hours after inoculation, and we have also done this in the majority of cases. In the presence of a positive reaction, however, a clear-cut picture can often be obtained after forty hours and if not *in vivo*, then certainly in the alcohol bath (see below). In examining the cornea, one should bear in mind that the smallpox virus does not produce a keratitis by this time. One does not see any opacities, but merely little dew-drop-like elevations which, in contradistinction to dust particles, with which they may be confused at times, are not movable when the eyelids are opened and closed. At this time already, some of the little blebs may present crater-like depressions.

Examination of the Enucleated Eyeball in Sublimate Alcohol.—In spite of the fact, that with experience a positive reaction may be recognized *in vivo*, we would recommend that the findings at this time, whatever they may be, be controlled by an examination in the alcohol bath. To this end the animal is killed by cervical shock and subsequently bled from the cervical vessels, so as to avoid bleeding during the enucleation of the eyeball. Both eyeballs are removed and immediately placed in a glass tray containing sublimate alcohol, taking care that the solution covers the eyes. The latter is composed of two volumes of a saturated solution of mercuric chloride and one volume of ordinary grain alcohol (94 to 96 per cent). The mercuric chloride solution is prepared by dissolving 4 grams of the salt in 60 c.c. of water, with the aid of heat, and then cooling and filtering. The examination should always be made against a black background and continued for from two to four minutes, by which time a maximum differentiation of the picture will have been obtained. At the expiration of that time the specimens are removed to 70 per cent alcohol, where the resulting appearances may be studied at leisure and photographed if desired.

Appearance of the Smallpox-infected Cornea and the Noninfected Cornea, after Treatment with Sublimate Alcohol.—In the sublimate alcohol bath, the control cornea becomes uniformly opaque, except for the lines of scarification

which occasionally are still visible. Not infrequently a slightly mottled appearance develops at first, but this gradually changes to a uniform opacity.

A smallpox-infected cornea presents an entirely different appearance. The general background here also is evenly opaque, but here and there within the area of scarification, and usually along the lines of the scratches and at



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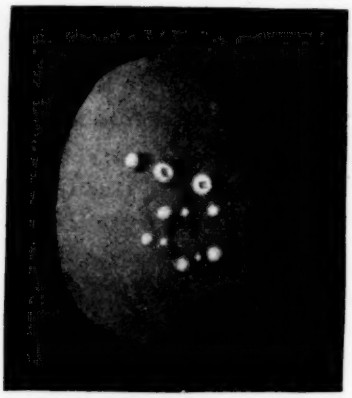

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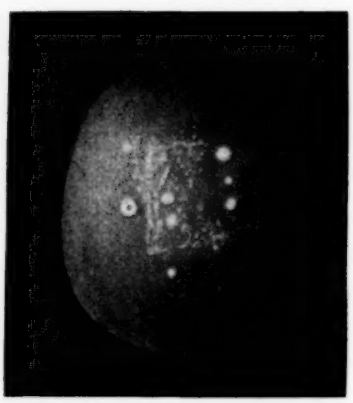

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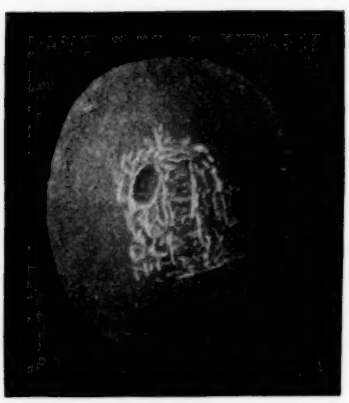


4

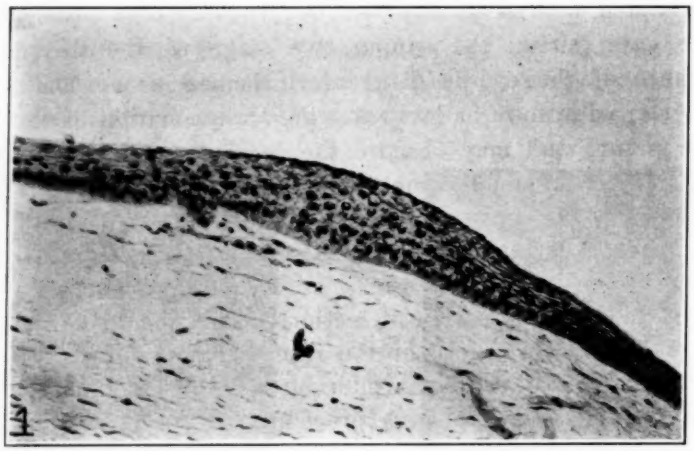
Plate I.

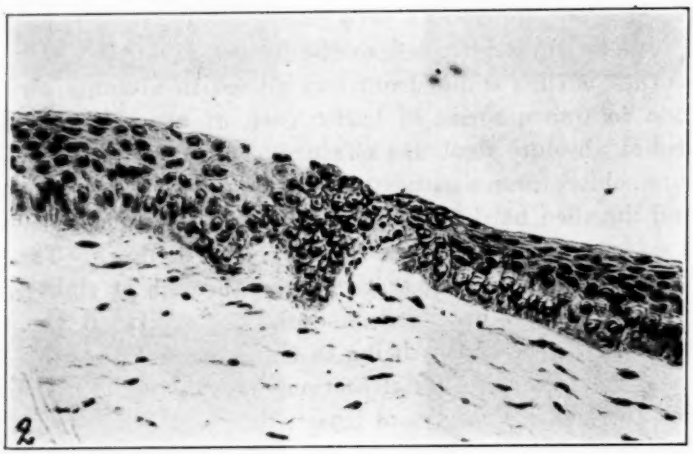
the points of intersection, *intensely white and opaque* little elevations make their appearance, some of which, even at this time, show crater-like depressions. These elevations correspond to the little dew-drop-like blebs which were seen *in vivo*. The number of elevations may vary from two or three to a dozen or more. They are usually discrete in outline, with clear-cut margins, though at times neighboring ones may be confluent. Not infrequently they occur in "string-of-pearl formation" along the lines of scarification.

Somewhat confusing pictures may present themselves when the variola virus is associated with large numbers of pyogenic organisms, as is so frequently the case during the suppurative stage of the disease. *In vivo* the appearance of the eye is different, inasmuch as corneal opacities of variable degree, with more or less extensive desquamation of the epithelium, control the picture and may obscure the focal changes due to the variola virus *per se*. There is then also more or less conjunctivitis with a corresponding exudation of pus. In the sublimate bath, however, it is almost always possible to reach a correct diagnosis, as some of the variolar blebs will be recognizable as such (Plate I, Fig. 3). When in doubt, of course, it is best to resort to the examination of sections. This should be done as a matter of routine and irrespective of the macroscopic findings, unless the observer has already acquired a considerable amount of experience. To this end Paul recommends the following procedure:

Histologic Technic (Rapid Method of Paul).—If the eyeball was examined in 70 per cent alcohol, after a preliminary sublimate alcohol bath of from two to four minutes, it is returned to the latter for approximately ten minutes. The cornea is then snipped out and placed in absolute alcohol for one minute. Then follows a series of baths, each of one minute's duration, in iodinized alcohol, absolute alcohol, a mixture of equal parts of absolute alcohol and chloroform, chloroform, a saturated solution of hard paraffin in chloroform, at 40° C., and liquefied hard paraffin at 60° C. The cornea is now embedded in the usual manner, with its convex side either up or down. The block, after being permitted to harden in ice water, is cut through at right angles to the cornea; the two halves are up-ended, mounted and sectioned, the idea of division and up-ending of the cornea being to obtain sections passing through the lesions, as soon as possible. Serial sections are mounted in the usual manner, "blotted" with several layers of lense paper that has been well moistened with absolute alcohol, and placed in the drying oven at 60° C. for a few minutes. The sections are next deparaffinized in xylol, run down through the alcohols, stained for five minutes in hemalum, run through the alcohols upwards, cleared in xylol, and mounted in balsam. This is the technic which Paul recommends. In practice we have not obtained satisfactory results in this manner, and prefer to *proceed a little more leisurely,* particularly with the processes of dehydration and infiltration with paraffin. The allowance of more time will give more satisfactory pictures and do away with the necessity of redehydration and reembedding which overhaste only too often requires.

The Histologic Picture of the Variola-infected Cornea.—In sagittal sections the most striking features are the epithelial hummocks (Plate II, Fig. 1) which correspond to the little protuberances that one sees in the sublimate alcohol bath or in the living eye. These hummocks, at this stage of the process, are the result of an increase in the size of the epithelial cells, due to the imbibition of fluid. The basal cells become involved first, and in consequence of their increase in both length and breadth, they are thrown out of their normal position in reference to one another, as well as to the overlying cells, which then also undergo hydropic changes. A picture thus results which





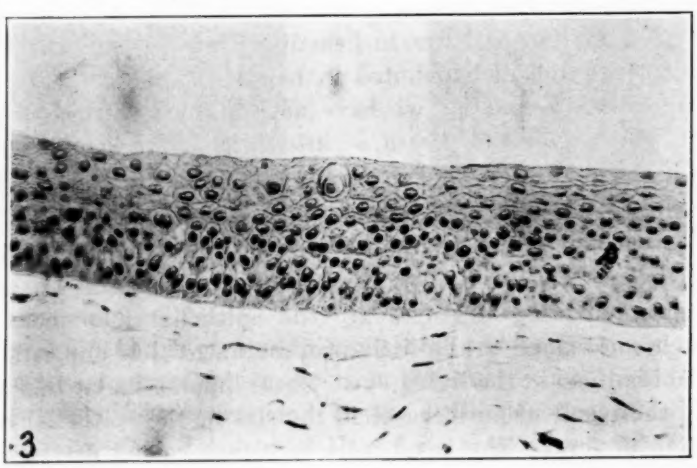


Plate II.

suggests a far more extensive degree of stratification of the cells than is normal for the cornea. The breadth of the epithelial layer in the center of the hummock is almost twice that of the normal peripheral zone. The pathologic process evidently extends peripherally, for the most advanced changes are encountered in the center of the hummock, and it is in this portion that desquamative changes first take place, with the consequent production of crater-like defects. With beginning central desquamation so-called box cells (Schachtelzellen) frequently appear in this area (Plate II, Fig. 3), and it is here also that the Guarnieri bodies are most numerous (Plate II, Fig. 2). The cells in question were first described by Hückel (1898) and named Schachtelzellen to denote their supposed origin, one cell becoming boxed up in another, as the result of pressure invagination, and the pair in turn in yet another, and so on.

The Guarnieri bodies, as seen in sections, are of variable size, ranging from a micron to about one-third the size of the nucleus of the epithelial cell. They are usually round, oval or lenticular, but not infrequently a little irregular in form. It is generally stated that they stain with nuclear dyes. This is true, when nuclear dyes only are used, but when a mixture of a nuclear dye and an acid dye is employed, such as eosin and hematoxylin, they present a mixed tone, in which the affinity for the acid dye seems to be more pronounced. Paul lays special stress on the prime importance of the character of the epithelial lesions, and regards the presence or absence of the Guarnieri bodies as of secondary value, from a diagnostic standpoint. He emphasizes also that the search for them may be a tedious one, and that they appear only in larger numbers at a relatively late stage. We are quite willing to admit the specificity of the epithelial changes and their diagnostic value, when they are clear-cut, but it may happen that these changes are not sufficiently pronounced to serve alone as criteria for diagnosis. In such cases the search for Guarnieri bodies is imperative, and their presence must be viewed as deciding evidence in favor of a positive diagnosis. In searching for them it should be borne in mind that they occur only in the diseased areas, viz., in areas where the normal arrangement of the cells has evidently been upset. It is true that these bodies appear in larger numbers later in the development of the lesions, but there can be no question that at the time when the animal is usually killed for the Paul test, i.e., forty-eight hours following inoculation, they are not so scarce that they are difficult to find.

Personal Observations.—In our first paper[4] we reported our findings in the course of the examination of 104 corneas. Since then the number of individual examinations has reached 247. The series now comprises forty-five examinations following inoculation with pustular material from active cases of smallpox; twelve examinations following inoculation with nasopharyngeal swabbings from smallpox convalescents; twenty-seven inoculations with vesicular fluid and eleven with nasopharyngeal swabbings from cases of chickenpox; sixty-seven normal controls of which nineteen had not even been scarified. In addition, corresponding examinations were made in twelve cases of herpes simplex; nine of herpes zoster; two of herpes genitalis; five of pem-

phigus; one each of dermatitis herpetiformis and ringworm, besides a number of examinations after inoculation with streptococci and staphylococci.

Our results bear out the validity of Paul's claim that the method is a dependable one and well suited for purposes of diagnosis.

In forty-four of the forty-five cases of bona fide smallpox the macroscopic reaction was in itself sufficient to reach a diagnosis. In only one was the result noted as "doubtful," but in this instance, as in all others in which sections were examined (thirty-nine), the histologic picture was a clear-cut positive for smallpox. In none of the varicella cases or any of the various skin affections which were examined was a reaction noted that even remotely was suggestive of a positive reaction. The histologic picture in these cases is, of course, also very different. In no instance were epithelial hummocks noted or any cell inclusions which in the least resembled the Guarnieri bodies. In the case of herpes simplex, herpes genitalis, dermatitis herpetiformis and pemphigus, there were markedly destructive lesions, with extensive epithelial desquamation, while in herpes zoster neither a conjunctivitis nor a keratitis was observed; in none of the nine cases of herpes zoster, moreover, were cell inclusions of any kind encountered.

PERSISTENCE OF THE SMALLPOX VIRUS IN THE NASOPHARYNX OF CONVALESCENTS

Friedmann and Gins[2] have pointed out that with the Paul method, the virus of smallpox may at times be demonstrated in the nasopharyngeal secretion of active cases and convalescents. According to their findings, the virus may indeed persist in the nasopharynx for several weeks.

During the past winter, through the courtesy of John Collinson of the Maryland State Board of Health, we had the opportunity to examine two cases of smallpox at the termination of the active stage of the malady and again at the time of the usual release from quarantine. In the one instance, a positive Paul reaction was obtained with the nasopharyngeal swabbings on the thirty-first day of the disease, and in the other on the twenty-third day, at which time the skin lesions had entirely disappeared.[5]

Control examinations with material from two normal persons and from nine cases of chickenpox in various stages of the disease, as well as from two chickenpox contacts, gave negative results. In three additional cases of smallpox, in which the swabbings had been taken respectively on the eighth, ninth and eighteenth days of the disease, the results were negative. It is noteworthy that in the two positive cases the process of swabbing was quite painful, while the three negative patients made no complaint, which suggests that in the first case two ulcerative processes were still going on.

CONCLUSIONS

1. The so-called Paul reaction, when controlled by histologic examination, constitutes a most valuable method for the diagnosis of smallpox.

2. In many cases the macroscopic picture of the inoculated rabbit cornea, in the sublimate alcohol bath, is in itself sufficient for diagnosis.

3. A negative macroscopic result will probably always warrant a negative diagnosis as to smallpox, if satisfactory material is available for the examination.

4. As in other diagnostic laboratory tests, the validity of the results will depend in part upon the experience of the worker and the technic employed in procuring the material for examination.

5. In chickenpox, negative results are so constant that a doubtful reaction should arouse suspicion and call for a further examination.

6. Pyogenic infections of the cornea *per se* do not interfere with the reaction.

7. The smallpox virus was found in one instance to retain its activity in the dried-drop preparation, at ice box temperature, for 387 days.

8. Before release from quarantine a careful rhinoscopic examination should be made in all smallpox cases, and followed, if need be, by the application of the Paul test to the nasopharyngeal secretion.

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AN IMPROVED COLORIMETER*

By John A. Killian, Ph.D., New York City

THE rapid development of colorimetric methods within the past decade testifies to the practicability of these quantitative procedures in biochemistry; however, their general simplicity may tempt the unwary to sacrifice accuracy for speed. That the analyst should be familiar with all the fundamental chemical reactions upon which the analytical methods have been based, has frequently been emphasized. Moreover, it is equally essential for maximum accuracy that the colorimetry be carried out under standard conditions, particularly of light. This need presents a problem in the average hospital and students' laboratories. The qualities of daylight are too variable and artificial daylight lamps are both cumbersome and costly, and the source of light is not fixed in relation to the solutions being matched. The improvements introduced in the colorimeter described, we find, satisfy this need in our everyday work. The instrument is not entirely new, it has been constructed by utilizing the best mechanical parts of the Klett and Bock-Benedict colorimeters, with a few additional improvements.

The general appearance of the colorimeter is shown in Diagram I. It is a plunger type of colorimeter, weighing about 2.3 kilograms and standing 30 cm. high. The instrument occupies but a small amount of space and may be read with comfort on a laboratory desk of average height. The cups are of black glass with overflow tops and fused on clear glass bottoms. Similarly the cylindrical plungers are of black glass with clear glass bottoms fused on. In this union of the clear glass to the black glass, simple fusion without a flux has been employed. The repeated use of mixtures of chloroform, acetic anhydride and sulphuric acid, such as are necessary in cholesterol estimations, have not injured the cups or plungers. The diameter of the plungers is 1.1 cm., that of the cups 1.4 cm., and but 4 c.c. of fluid are required to fill the cups conveniently for reading.

The cups are supported on mounted stages by rack and pinion on separate nickel-plated pillars. The rack and pinion are constructed of brass and operate on a spring system. This mechanism has been found, in the Bock-Benedict colorimeter, to minimize the lost motion due to wear after continued use. The light from the plungers is reflected into the eyepiece by Helmholtz prisms. On the posterior portion of the prism housing, a mirror placed at an angle of 45° facilitates the reading of the vernier scale. The zero point of the scale on both pillars is readily adjustable by a thumb screw. The reading mirror and vernier scale are similar to those of the Klett colorimeter. The base measures 14 by 11 cm. and is relatively heavy so that the instrument is un-

*From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital.

usually well balanced. On the top of the base there is a ground glass mirror for use in daylight. One border is fixed to the base and the mirror may be moved through an arc of 90° and firmly secured at any position by an adjustable screw. Turning this mirror forward at a right angle with the base discloses a built-in substage lamp for artificial daylight. This substage lamp is composed of a glass tubular electric bulb with a frosted surface. A long slender filament extends around the entire periphery assuring a uniform intensity of light from the entire surface area of the bulb. The electric bulb (25 Watt) is covered by a blue glass of good neutral color. The fact that both cups are receiving equal intensities of light is ensured by the fixed

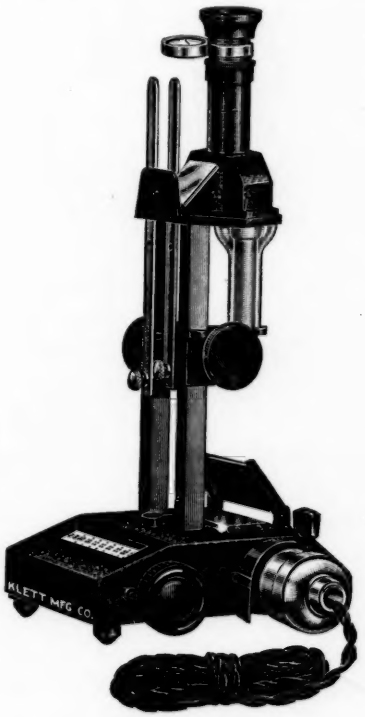

Diagram I.

position of the daylight lamp in relation to the cups, which renders this instrument far superior to any other colorimeter with artificial daylight. In fact it has been found that the color comparisons are more uniform with the substage lamp than with the varying qualities of daylight in the average laboratory. The lamp is readily turned on or off by means of a switch at the right-hand side of the base. It appeared possible that the temperature of the solutions in the cups might be influenced by the use of this lamp, but there was noted no rise in temperature of solutions in the cups during a period of ten minutes continuous illumination with the lamp. There is no necessity, however, for using the lamp except during the period of color comparison.

The posterior portion of the base carries tables facilitating the calculation of results. The tables are printed in black on a linen sheet, which is

operated by a hand wheel. Switching on the substage lamp also illuminates the tables. Since all color comparisons are made with the standard set either at 15 or 20 mm., the calculations for $\frac{15}{R}$ and $\frac{20}{R}$ have been tabulated. The recorded results are for readings for the unknown between 6 and 37, and have been calculated to the first decimal place. The method of making a reading and of utilizing the table of calculations is illustrated in Diagram II which gives the actual size of figures. In any series of calculations, the two unknown factors are the reading and the dilution. The strength of

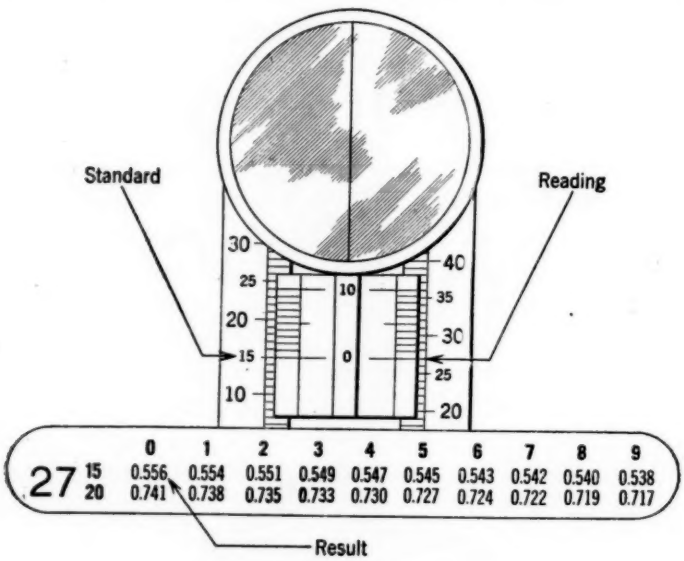

Diagram II.

the standard is a constant. Every equation will have this general form $\frac{15 \text{ (or } 20)}{R} \times s \times \text{dil} = \text{result}$. The result of the fraction $\frac{15 \text{ (or } 20)}{R}$ is determined immediately from the table. For example, if the standard has been placed at 15, and the reading is 27, reference to the table shows that $\frac{15}{27}$ is 0.556. The figures from 0 to 9, across the top of each section of the table represent the first figures to the right of the decimal point in the reading. Although the table presents calculations for readings from 6 to 37, to insure accurary, readings should not be made below 10 nor above 20 when the standard has been set at 15, and not below 15 nor above 25 when the standard has been set at 20.

A separate table is provided for the calculations of the hemoglobin of the blood. In the method described by Myers 0.05 c.c. of blood are added to 20 c.c. of 0.1 N HCl and thoroughly mixed. After standing at least four hours, the hematin hydrochloride thus formed is compared with a standard disc. In the colorimeter described the disc is fitted into a brass shoulder.

The plunger on the left hand side is unscrewed from the prism housing, and the disc with its shoulder fits into the space provided in the brass cap at the top of the plunger. The plunger is then screwed back into place. The cup on the left is about three-quarters filled with water and set at 10. The unknown on the right hand side is compared with this standard. The reading is noted, and a reference to the table gives the grams of hemoglobin in 100 c.c. of blood corresponding to the reading. For this result the following formula has been used: $\frac{10}{R} \times 0.38 \times 400 =$ per cent of hemoglobin. The intensity of color in the disc corresponds to 0.038 per cent of hemoglobin in 0.1 N HCl placed at 10 in the colorimeter.* For example, if the unknown read 10.5, it is evident from the table that the specimen contains 15.2 grams of hemoglobin per 100 c.c., i.e., 15.2 per cent. For the proper interpretations of the results of blood analysis, an accurate knowledge of the hemoglobin concentration is indispensable. If the hemoglobin is unusually low 0.1 c.c. of blood may be used in place of 0.05 c.c., and a correction is made by dividing the final result by 2. When the hemoglobin disc is not in use, it is securely and conveniently held in a small brass cup on the base.

The telescopic eyepiece has one lens, permitting an adjustment of the focus through a wide range. The small aperture in the diaphragm of the eyepiece limits a well-defined field, as illustrated in Diagram II. When the dividing line between the two halves of the field is brought into sharp focus, the periphery of the field is also sharply focussed. On the posterior portion of the eyepiece there is attached a bifocal lens; the anterior half of this lens magnifies the image of the vernier scale in the reading mirror, the posterior half magnifies the calculation tables on the base of the colorimeter.

The colorimeter described presents some points of superiority over the instruments now in use. The substage lamp fixed in the base renders it possible to use the colorimeter anywhere and at any time in the laboratory. It is small, compact, and, hence, occupies little space. The calculation tables are an invaluable aid in a long series of calculations. The lenses permit reading the vernier and tables with accuracy and comfort.

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*This disc has been calibrated against hemoglobin of blood specimens determined by the oxygen capacity method.

SIMPLIFIED COLORIMETRIC CALCULATION*

By William H. Stoner, A.M., Phar.D., M.D., Philadelphia, Pa.

FOR the past eight years these laboratories have employed a simplified method of colorimetric calculation which, at the time of its introduction, seemed too obvious to justify its publication. Since that time, however, so many cumbersome tables,[1, 2, 3] graphs,[4] nomographs, special slide rules, etc., have been devised, complicating a most simple arithmetic process, that it has been suggested that the simple manipulatory artifice for avoiding routine colorimetric calculation be made known. Casual mention of the method has been published.[5] No doubt many laboratories are using an identical method, but, judging from many recent articles and textbooks, there are many workers in colorimetry who could reduce their routine calculations by the use of this method.

Just as factor solutions are employed to eliminate calculation in routine volumetric analysis, so can factor settings of the colorimeter be so chosen as to make the results of routine determinations derivable by inspection. Thus, the calculation of a routine determination may be made initially, once and for all, not only saving time but also entirely precluding the error inherent in the chemical technician's arithmetic.

The usual recommendation[6-9] for colorimetric work is to set the standard solution (in the left cup for a right-handed operator) at some fixed point, as 10 or 15 mm. depth, and to adjust the unknown solution until color equality is obtained. This necessitates division by the reading of the unknown. A simple reversal of conditions eliminates this division. Instead of fixing the standard solution at a definite reading and adjusting the unknown solution to color equality, the standard solution (in the right cup for a right-handed operator) is adjusted to match the unknown solution set at some fixed point so calculated that the reading of the standard becomes the sought result of the determination or some simple multiple or submultiple thereof.

Probably the most useful and well-known field for exemplification of this manipulatory evasion of routine calculations is that of clinical blood chemistry. Similar methods may be applied to all colorimetric determinations.

Following is the method of determining a factor colorimetric depth of solution derived from blood so as to obviate paper and pencil, nomographic, logarithmic, slide rule or tabular routine calculations. The method is here applied to the Folin and Wu[10] determinations of dextrose, total nonprotein nitrogen and creatinine, to the Benedict[11] uric acid determination, to the Myers and Wordell[12] cholesterol determination and to the Briggs[13] determination of inorganic phosphorus. A general method is given in order that sim-

*From the Biochemistry Laboratories of the Philadelphia General Hospital and of the Graduate School of Medicine of the University of Pennsylvania.
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ilar reasoning may be applied readily to all other colorimetric determinations, as has been done in these laboratories. Letters have been assigned the various values merely that the demonstrations for the six determinations may be shown compactly in tabular form.

Let x = mg. of sought constituent in 100 c.c. of blood (the conventional clinical method of expression of results of chemical blood analyses).
b = reading (in mm.) on colorimeter of solution derived from blood.
s = reading (in mm.) on colorimeter of solution containing standard.
V = total c.c. of colorimetric solution containing standard.
v = total c.c. of colorimetric solution derived from blood.
C = mg. of sought constituent (standard substance) in v c.c. of the colorimetric solution containing standard.
c = mg. of sought constituent in the total volume of colorimetric solution derived from blood.
B = total c.c. of blood sample represented by the colorimetric solution derived from blood.
Since c = mg. of sought constituent in B c.c. of blood, the mg. of sought constituent in 100 c.c. of blood, or

$$x = \frac{100\ c}{B} \quad \text{(1)}$$

From the universal colorimetric proportion (Beer's law).

$$C : c = b : s \text{ or}$$

$$c = \frac{C\ s}{b}$$

Substituting this value of c in (1),

$$x = \frac{100\ Cs}{B\ b}$$

Let $$K = \frac{100\ C}{B}$$

When $$x = \frac{K\ s}{b}$$

The value of K in each particular analysis determines the most convenient setting of the colorimeter scale for the colorimetric solution derived from blood.

Table I.—From the values of x, in terms of the readings of the adjusted standard solution, in the last column of the table it is apparent that the milligrams of constituent per 100 c.c. of blood may be read from the colorimeter scale either directly as in the case with nonprotein nitrogen, or by such simple processes as changing the position of a decimal, or multiplication or division by 2, 5, etc.

It has been suggested by Walter G. Karr and Bernard L. Oser, whose experience in the instruction of internes and technicians is large, that this method would be more useful and more easily understood if literal values for the various quantities especially of K, were avoided. Accordingly they use the practical teaching formula given on page 576.

It is seen that the mg. of constituent per 100 c.c. blood is the product of four factors, designated for brevity, (1) the colorimetric factor, (2) the standard factor, (3) the sample factor and (4) the dilution factor. When the

final colorimetric solutions are equal in volume, the dilution factor, (4) is (1) and may be ignored.

The values of the terms of this formula given in the original methods are modified in some cases, cholesterol for example, in these laboratories for

$$\left(\begin{array}{c}\text{Mg. of constituent}\\ \text{per 100 c.c. blood.}\end{array}\right) = \frac{\left(\begin{array}{c}\text{Reading of}\\ \text{standard}\end{array}\right)}{\left(\begin{array}{c}\text{Reading of}\\ \text{unknown}\end{array}\right)} \times \left(\begin{array}{c}\text{Mg. of standard}\\ \text{in colorimetric}\\ \text{solution}\end{array}\right) \times \frac{100}{\left(\begin{array}{c}\text{C.c. of blood used}\\ \text{for colorimetric}\\ \text{solution}\end{array}\right)} \times \frac{\left(\begin{array}{c}\text{C.c. of unknown}\\ \text{colorimetric}\\ \text{solution}\end{array}\right)}{\left(\begin{array}{c}\text{C.c. of standard}\\ \text{colorimetric}\\ \text{solution}\end{array}\right)}$$

$$\left(\begin{array}{c}\text{Mg. of dextrose}\\ \text{per 100 c.c. blood.}\end{array}\right) = \frac{\left(\begin{array}{c}\text{Reading of}\\ \text{standard No. 1}\end{array}\right)}{20} \times 0.2 \frac{100}{0.2} \times \frac{25}{25} = 5 \times \text{Reading of standard No. 1.}$$

$$\left(\begin{array}{c}\text{Mg. of nonprotein}\\ \text{nitrogen per 100}\\ \text{c.c. blood.}\end{array}\right) = \frac{\left(\begin{array}{c}\text{Reading of}\\ \text{standard}\end{array}\right)}{30} \times 0.3 \frac{100}{0.5} \times \frac{50}{100} = \text{Reading of standard.}$$

$$\left(\begin{array}{c}\text{Mg. of Creatinine}\\ \text{per 100 c.c. blood.}\end{array}\right) = \frac{\left(\begin{array}{c}\text{Reading of}\\ \text{standard}\end{array}\right)}{30} \times 0.03 \frac{100}{1} \times \frac{15}{30} = 0.05 \times \text{Reading of standard.}$$

$$\left(\begin{array}{c}\text{Mg. of uric acid}\\ \text{per 100 c.c. blood.}\end{array}\right) = \frac{\left(\begin{array}{c}\text{Reading of}\\ \text{standard}\end{array}\right)}{20} \times 0.02 \frac{100}{0.5} \times \frac{15}{15} = 0.2 \times \text{Reading of standard No. 1.}$$

$$\left(\begin{array}{c}\text{Mg. of cholesterol}\\ \text{per 100 c.c. blood.}\end{array}\right) = \frac{\left(\begin{array}{c}\text{Reading of}\\ \text{standard}\end{array}\right)}{16} \times 0.4 \frac{100}{0.25} \times \frac{7.1}{7.1} = 10 \times \text{Reading of standard.}$$

$$\left(\begin{array}{c}\text{Mg. of phosphorus}\\ \text{per 100 c.c. blood.}\end{array}\right) = \frac{\left(\begin{array}{c}\text{Reading of}\\ \text{standard}\end{array}\right)}{12.5} \times 0.05 \frac{100}{1} \times \frac{9}{18} = 0.2 \times \text{Reading of standard.}$$

As an aid to the memory for rapid routine work Table II is given.

the purpose of deriving more convenient factor colorimetric settings. To avoid confusion of the method, however, in the following formulas the original values are retained.

TABLE I

NUMERICAL DATA FOR CALCULATING SIMPLIFIED FACTOR FORMULAS FOR THE COLORIMETRIC DETERMINATIONS OF CHEMICAL CONSTITUENTS OF BLOOD

CONSTITUENTS	V	v	C		B	K		x		b		x	
			STANDARD 1	STANDARD 2		STANDARD 1	STANDARD 2	STANDARD 1	STANDARD 2	SETTING 1	SETTING 2	SETTING 1	SETTING 2
Dextrose	25	25	0.2	0.4	0.2	100	200	$\frac{100\ s1}{b}$	$\frac{200\ s2}{b}$	10	20	$\frac{10\ s1}{20\ s2}$	$\frac{5\ s1}{10\ s2}$
Nonprotein Nitrogen	100	50	0.15		0.5	30		$\frac{30\ s}{b}$		30	15	s	2 s
Creatinine	30	15	0.015		1	1.5		$\frac{1.5\ s}{b}$		30		0.05 s	
Uric Acid	15	15	0.02	0.05	0.5	4	10	$\frac{4\ s1}{b}$	$\frac{10\ s2}{b}$	10	20	$\frac{0.4\ s1}{s2}$	$\frac{0.2\ s1}{0.5\ s2}$
Cholesterol	7.1	7.1	0.4		0.25	160		$\frac{160s}{b}$		16		10	
Phosphorus	18	9	0.025		1	2.5		$\frac{2.5s}{b}$		12.5		0.2	

TABLE II

FACTOR SETTINGS OF COLORIMETRIC SOLUTIONS DERIVED FROM BLOOD, TOGETHER WITH THE CORRESPONDING NUMBERS BY WHICH THE READINGS OF THE STANDARDS ARE MULTIPLIED TO OBTAIN THE MG. OF THE RESPECTIVE CONSTITUENTS PER 100 C.C. OF BLOOD

CONSTITUENT	DEXTROSE	NONPROTEIN NITROGEN	CREATININE	URIC ACID	CHOLESTEROL	PHOSPHORUS
Set unknown at	20	30	30	20	16	12.5
Multiply reading of standard (#1) by	5	1	0.05	0.2	10	0.2

SUMMARY

The simple, and universally applicable, manipulatory method of obviating routine colorimetric calculations with their accompanying errors by setting the unknown colorimetric solution at a fixed depth and adjusting the standard solution to color equality is illustrated by six examples in blood chemistry.

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THE DETERMINATION OF GLOBULIN INCREASE IN SPINAL FLUID*

BY GEORGE E. ROCKWELL, M.D., M.A., CINCINNATI, OHIO

THE tests in general use for globulin increase in spinal fluid are: Noguchi's butyric acid method, the stratification with saturated ammonium sulphate solution, or Pandy's carbolic acid method. Noguchi's method is by far the better, but it is objectionable because of the butyric acid odor. The ring test with saturated ammonium sulphate, is too slow in appearing to give complete satisfaction, while Pandy's carbolic acid method does not give detailed information about the amount of increase of globulin, and the border line between the normal amount and increased amount, is not clear-cut.

I have found that the following solution which is a modification of Robert's solution (used for testing urine for albumin), is of very great value in the testing of spinal fluid for increased globulin. The solution consists of one part concentrated nitric acid, four parts of water which has been saturated with both magnesium sulphate and sodium chloride.

*From the Department of Bacteriology and Hygiene of the University of Cincinnati.
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The test is performed by adding in a small test tube, one-half to one cubic centimeter of spinal fluid; then one cubic centimeter of the reagent is introduced with a pipette, placing the pipette at the bottom of the tube and allowing the reagent to run in under the spinal fluid. A definite line of demarkation can be seen between the reagent and the spinal fluid and if there is any increase in globulin, a cloudiness will immediately appear in the form of a ring. This cloudy ring can be broadened by gently agitating the tube. The amount of increase is indicated by the size and intensity of the ring.

The value of this test for increased globulin in the spinal fluid over the older methods is that there is no objectionable odor; it reacts quicker and better than ammonium sulphate test and the line of demarkation between a normal amount and an increased amount of globulin, is clear-cut.

A DRYING APPARATUS FOR LABORATORY VESSELS*

By Joseph C. Bock, Ch.E., Ph.D., and Max Gilbert, Ph.D., Milwaukee, Wis.

THE most widely adopted method for drying the inside of glass vessels consists in rinsing with strong ethyl alcohol, and removing the residual alcohol by means of ether. An alternative is the drying in an air oven. The alcohol-ether procedure, while fairly rapid, is rather costly. Besides that, vessels so dried, usually do not drain perfectly, evidently on account of some slight impurities in the ether. Furthermore, it must be considered that pure ethyl alcohol cannot be used as freely as it was used a few years ago. The drying in an oven is a rather time-consuming process.

It is the purpose of the present paper to describe an easily constructed, inexpensive drying apparatus. The arrangement as described has been used in this laboratory for over two months with very satisfactory results, and has been found superior to the above mentioned methods in every way.

The apparatus requires for its use compressed air and a Bunsen burner. The principle is simple. A stream of air is passed through a coil which is heated by a gas flame. The heated air escapes through one or several perforated tubes over which the vessels to be dried are suspended.

The dryer is made of standard one-eighth inch pipe and fittings which may be procured at any plumbing supply house. The construction is herewith described:

The heating coil is made first. This is done by joining two one-eighth inch, ninety degree elbows (technically termed "Ells") by means of a close nipple. Into one end of this U-shaped arrangement, another close nipple is inserted. On to this is screwed another elbow, then another close nipple, continuing this way alternatingly until the spiral has the desired length.

*From the Department of Physiological Chemistry, Marquette University School of Medicine, Milwaukee, Wisconsin.

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In the lower tiers of the coil the elbows are not brought together very closely, the distance between the flanges being about one-half inch. In the upper tiers the elbows are brought together more closely by giving a few more turns, so that the sides of the coil taper towards the top. The number of elbows used is best given by referring to the illustration. The left front corner has eight elbows, the right front corner seven, the left rear corner seven, and the right rear corner the same number.

Into the lowest elbow of the coil (seen at the lower left front corner), is screwed a four and one-half or five inch nipple; this is joined by means of a union to another four or five inch nipple. The free end of this is connected with the compressed air supply by a rubber tube.

To the last elbow on top is attached a U, made of two elbows and a close nipple, as illustrated. A six inch nipple is attached to this, then follows

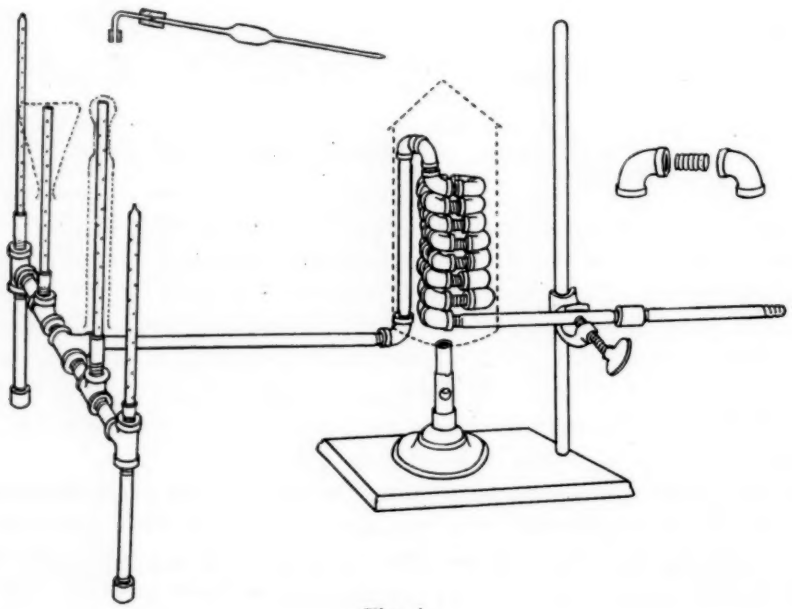

Fig. 1.

another elbow and another six inch nipple. Screw on to this a T. Into the free ends of the T are inserted short nipples (the short nipples are somewhat longer than the close nipples). There follows on each side a T, a short nipple, and another T. This last T is attached to the nipple by the middle opening. Three inch nipples, closed by caps, form the legs. The third point of support is a small ring stand, to which the intake pipe is held by an ordinary clamp holder.

Into the free openings of the four T's the drying tubes are inserted. These are made as follows:

A piece of one-eighth inch brass tubing is driven into a close nipple, the outside diameter of the brass tubing being slightly larger than the bore of the nipple. When doing this, the nipple should rest on a piece of hard wood, not on metal, otherwise the thread will be spoiled. It may happen that

the brass tube has a rather loose fit. In this case, the upper end of the nipple is pinched in a vise, thereby tightening on the tube. If an air-tight union is desired (desirable but not imperative), a little solder should be applied around the upper end of the nipple. The brass tubes may be of varying length, depending on the vessels that are usually dried. We prefer tubes about nine inches long. When only small tubes and flasks are to be dried, shorter tubes are advisable. Several holes are drilled into the tubes allowing the heated air to come in contact with the walls of the vessel in several places. To obtain the best results, these holes are made with a very small drill (size 54-60), the holes being drilled straight through, each pair of holes about 10 mm. distant from the next. The line of one pair of holes is at a ninety degree angle to the next. The opening on top is narrowed to a small slit by pinching the end of the tube in a vise or clamp. The drying tubes are screwed into the openings of the T's by hand and gently tightened with pliers. They should not be screwed in too tight, otherwise it may be found very difficult to remove them later on.

For heating, we use a Bunsen burner sawed off to fit under the coil. The coil is covered with a short piece of small stove pipe, or by wrapping asbestos paper around it, holding the asbestos in place with wire. The top of the covering is closed in a suitable way to prevent undue loss of heat.

When using the dryer, the coil is first heated for a few minutes with the air turned on. The apparatus is now ready for use. The vessels to be dried are hung over the tubes. When short test tubes or small flasks are dried, the lower part of the drying tubes may be closed, if desired, by slipping a loose fitting sleeve over it. This sleeve is made from a piece of hollow curtain rod of appropriate size.

For the drying of pipettes the following arrangement has been found effective. A piece of the brass tubing, about three inches long, is joined to a nipple in the manner described above. The upper end of the tube is now heated to a red heat, the end, which is attached to the nipple, being held in a piece of wet cloth. The tube may be bent very easily after it is cold. It is bent over until the end points slightly downward. The tube is screwed into one of the openings. A hole large enough to accommodate the pipe is bored in a cork, the cork is slipped half way into the drying tube. Into the other end the pipette is inserted, the tip of the pipette resting on a piece or pad of filter paper on the table on which the dryer stands.

When drying tubes of very small diameter, or larger tubes with constrictions, such as Folin-Wu sugar tubes, it may be necessary to decrease the outside diameter of the drying tube by filing, or better, by turning down with a lathe. The drying of a small flask or a large tube, such as a Folin-Wu sugar tube, is usually accomplished in a minute or less. Smaller tubes are dried in about thirty seconds.

The apparatus can be made for less than four dollars, if a drill press is available, otherwise the cost for drilling the small holes in the drying tubes has to be added.

SUMMARY

An inexpensive, easily constructed apparatus for the drying of glass vessels by heated air is described.

A NOTE UPON A METHOD FOR THE PREPARATION OF ANTISHEEP HEMOLYSIN*

By Robert A. Kilduffe, A.B., A.M., M.D.

In a previous communication[1] concerned with the preservation of antisheep hemolysin the following statement appears:

"The preparation of antisheep hemolysin of high titer is such a relatively simple and inexpensive procedure that, if its preservation over long periods of time can be assured, there is little reason for the use of relatively low titer commercial preparations."

This statement was based upon practical experience in the preparation of high titer (1:15,000 to 1:40,000), antisheep hemolysin extending over a long period, during which time success has been practically uniform; but, since its publication, so many inquiries have been received as to the details of the method used that, apparently, the degree of success attained has not been the usual experience of others. In response to these inquiries, therefore, the present note detailing the method used is herewith presented.

In so far as I know, the method embodies nothing original. It is based upon that published by Kolmer,[2] although, upon consulting this reference, I find that there are minor points of difference which, however, do not appear to be of marked significance. As the method has given complete satisfaction, it is reported in full detail.

The Animal.—Healthy, adult rabbits of good size; males or females may be used but pregnant females are avoided. No special breed is necessary, nor any special diet. The animals are simply kept under ordinary conditions in clean cages.

The Antigen.—Sheep cells are used for injection on the day on which they are collected. Where a sheep is not maintained in the laboratory, abattoir cells may be used. Abattoir blood should be collected directly from the animal as it is killed, in a sterile bottle containing sufficient 5 per cent sodium citrate solution made up in normal saline to prevent coagulation. The sodium-citrate solution may be kept on hand but should be sterile and kept in a sterile bottle.

The cells are washed in normal saline in the centrifuge for six washings at high speed, being well packed each time. After the sixth washing the supernatant fluid should be tested for albumin by the heat and acid test to avoid the possibility of anaphylactic reactions in the rabbit. Should albumin be found, which has never occurred after the sixth washing, the washing should be continued until the fluid is albumin free.

Under sterile precautions, using a sterile pipette and sterile test tubes,

*From the Laboratories of the Atlantic City Hospital.
Received for publication, August 5, 1924.

to one centimeter of the packed cells is added nine cubic centimeters of sterile normal (0.85 per cent) saline solution, thus making a 10 per cent suspension.

The Injection.—The rabbit is removed from the cage and its body wrapped in a towel, leaving the head and ears free. An animal box may be used if desired. The hair is removed from the ear by means of a depilatory paste (one part of barium sulphide and three parts of starch), or a commercial depilatory may be used. After the paste has been thoroughly washed off with water, the ear is washed with alcohol, which is then removed with several spongings with water. The depilatory paste renders the marginal vein easily seen and quite prominent, by reason of congestion, so that the injection is facilitated. For future injections the ear is simply washed with alcohol and water. If the vein is very small it may be made swollen and prominent by tapping with the finger or sponging with xylol. If xylol is used it must be thoroughly removed with alcohol to avoid an inflammatory reaction.

The syringe, of the Luer type, is sterilized by dry heat together with the needle, which should not be of too small a caliber. Ten cubic centimeters of the 10 per cent cell suspension are injected at a time.

After the cells have been injected, a cotton compress is held over the site of the injection for a minute or two and the animal returned to the cage.

The injections should begin as far forward in the ear as is possible in order that a new portion of the vein toward the base of the ear may be used for each subsequent injection.

Number of Injections.—Cells are injected—ten cubic centimeters each time—twice weekly (every three days). The injections should be made regularly on the appointed day. One week after the last injection, the rabbit is bled from the ear, the serum separated, inactivated for thirty minutes at 56° C. and titered.

If the titer is satisfactory, the animal is stunned by a blow at the base of the skull and bled into 50 c.c. sterile centrifuge tubes. Any method of bleeding may be used which is preferred. If the titer is not satisfactory one or two more injections may be given and the serum again tested one week after the last injection.

After its collection, the blood is allowed to stand overnight in the ice chest, the clot first being ringed, and removed by centrifugation.

Titration.—1. Stick an ear vein and collect one or two centimeters of blood in a small sterile tube. Allow to clot; ring the clot, and remove the serum by centrifugation.

Inactivate for thirty minutes at 56° C. In a large test tube place 0.1 c.c. of the serum and add 9.9 c.c. of normal saline, thus making a 1:100 dilution. Place one centimeter of this dilution in a large tube and add nine centimeters of normal saline, thus making a 1:1000 dilution, which is used for the titration.

2. Place twelve serologic test tubes in a rack and mark each with the amount of amboceptor dilution to be added as noted below.

To each tube add the amount of 1:1000 amboceptor dilution indicated: 0.05 c.c., 0.1 c.c., 0.15 c.c., 0.2 c.c., 0.25 c.c., 0.3 c.c., 0.35 c.c., 0.4 c.c., 0.45 c.c. and 0.5 c.c.

3. To the eleventh tube, which is the hemolytic control, add 2 c.c. of normal saline.

4. To the twelfth tube, which is the corpuscle control, add 1 c.c. of normal saline.

5. To each of the first ten tubes add 0.3 c.c. of a 1:30 dilution of complement and 0.5 c.c. of 2 per cent sheep cell suspension (one part of cells to forty-nine parts of normal saline). These amounts of cells and complement are used so that the titer of the amboceptor may be determined with reference to Kolmer's quantitative method of complement fixation.

To the hemolytic control add 0.3 c.c. of 1:30 complement dilution, 0.5 c.c. of 2 per cent cell suspension and two units of an amboceptor whose titer is known.

To the corpuscle control tube add 0.5 c.c. of sheep cell suspension.

6. Incubate in the water-bath at 38° C. for one hour. At the end of this time make a reading. The unit is contained in that tube which contains the smallest amount of amboceptor just giving complete hemolysis.

If hemolysis is complete in the tube containing 0.1 c.c. of amboceptor serum and not in the tube containing 0.05 c.c., the titer is 1:10,000 (0.1 c.c. of 1:1,000 dilution being equivalent to the same amount of amboceptor serum as 1 c.c. of 1:10,000). If hemolysis is complete in 0.05 c.c. the titer is at least 1:20,000. Under such circumstances, if it is desired to ascertain the exact titer, a new set up must be made with the following amounts of amboceptor serum: 0.01 c.c., 0.02 c.c., 0.03 c.c., 0.04 c.c., and 0.05 c.c.

The titer of the serum will, of course, be influenced by the amount and dilution of the complement dose. Prior to the publication of Kolmer's test, I used 0.5 c.c. of 1:20 complement.

Preservation of the Serum.—After its collection, the serum is inactivated in bulk for thirty minutes at 56° C. and re-titered as the titer is modified by the destruction of thermolabile amboceptors. It is then preserved by the addition of an equal volume of chemically pure glycerin. Two centimeters of the glycerinated serum, representing one centimeter of the serum alone, are placed in ampules which are then sealed for storage.

The above technic is simple and embodies nothing new as far as I am aware. I have had uniform success with it in the preparation of hemolysins of not less than 1:10,000 titer. As a rule, after four injections, the titer will approximate 1:20,000. On one occasion, after four injections the titer was 1:40,000 and on another occasion, after six injections the titer was 1:60,000.

It is well known, of course, that different animals will vary somewhat in their ability to produce hemolysin but success with the method has been so uniform that I seldom inject more than one rabbit at a time unless a large amount of hemolysin is desired.

On one occasion, I found a rabbit which, in spite of repeated injections, failed to give a satisfactory serum and I once encountered a sheep whose cells were very resistant to hemolysis. It is conceivable that such cells would not prove a good antigen; this, however, is an unusual occurrence and would be compensated for in subsequent injections unless the same sheep were used

throughout which would hardly be possible as the resisting quality of the cells would soon be noted in the course of the day's work and the animal discarded.

The results as stated which have been uniform and constant with the method outlined, have, as originally noted, made it unnecessary to rely upon relatively low titer commercial preparations of antisheep hemolysin.

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EDITORIALS

The Hospital Laboratory and the Resident

A VERY definite proportion of residents look upon the laboratory service as the least interesting of their hospital experiences, approach it with reluctance, and view its termination with feelings akin to enthusiasm and relief.

There is some tendency to attribute this to the natural preference of the average resident for the more dramatic and spectacular features of certain of the clinical services, but it is possible that the responsibility for this attitude, in some measure, may rest upon the director of the laboratory and his attitude toward the laboratory work and the laboratory resident with a consequent influence upon the character imparted to the resident laboratory service.

There are few, indeed, who are able to determine their natural aptitude and general fitness for specialization in the days of their hospital term, but

even if this were always possible, it may be taken as an axiom that no specialty is so distinct as to enable it to withdraw from contact with the practice of medicine as a whole, none which can neglect the keystone of intelligent treatment—diagnosis.

If the resident comes to look upon his laboratory service as something apart and distinct from all the other departments of the hospital; if it comprises simply so many blood counts, so many smears, or so many examinations of whatever kind completed and reported as entities, separate and distinct from the patient and his condition, it is easy to see that the laboratory service may soon become devoid of interest and productive of the usual effect of monotony. Under such conditions it is not surprising that the laboratory service is productive of little that the resident finds of value in his later clinical experience.

Routine examinations there must be and a certain proportion of them must fall to the lot of the resident and, of necessity, some of his time and the attention given him must be concentrated upon the acquisition of sufficient technical expertness to assure accuracy in his work.

It is, however, of value and certainly productive of increased interest in his work and conducive to his own and the benefit of his future patients, if an endeavor is made to focus attention upon the practical side of the laboratory service and upon its daily application to the scientific practice of medicine.

It may be accepted, as preliminary premises: that clinical laboratory procedures form an essential part of clinical surveys and study; that, to no small extent, these will be made for the clinician by those skilled and expert in laboratory methods; and that their ultimate value and significance will depend entirely upon their interpretation and application to the particular case.

It may also be assumed that the average medical graduate of today will be possessed, if not of sufficient skill to perform the laboratory examinations within his province, then certainly of a mental equipment which will suffice to enable him to find and absorb the information necessary.

It would seem logical, therefore, during the service of the laboratory resident to turn his attention, not so much toward methods and technic, as toward the interpretation and clinical significance of the examinations he helps to make or sees made in the laboratory.

It may be difficult to find much of interest in a series of blood counts, but interest is not only possible but, with the right mental attitude, inevitable if each count is appreciated and regarded as an examination into a particular phase of an individual patient; if it is appreciated that upon the blood count and its interpretation the diagnosis of operative interference may, at times, depend; that it may be utilized to detect or forewarn of impending complications or influence the prognosis.

The laboratory resident should be taught to realize and to appreciate that his laboratory service is intimately and definitely associated with the clinical side of medicine. He should be encouraged to assimilate from the chart or from those in clinical contact with the case the results of the survey of the patient already made through other means, and to correlate with these

the results of the work done in his own department; to determine by study, ratiocination, or inquiry, how these results may be applied to the clinical problem and, if they are not, apparently, capable of coordination with other findings, the probable reason and significance of the disagreement.

By this means and by such methods only will the student come eventually to estimate the true value of laboratory examinations as applied to the particular case; to assort his future requisitions intelligently for his own use and the benefit of his patients; to interpret them wisely, and to realize that laboratory examinations are not and cannot be pathognomonic; that they can be "standardized" only as to technic; and that the results depend, not upon the disease, but upon the reaction of the patient to the disease, a reaction influenced by all factors which may affect the individual capacity to respond to varied stimuli.

The director of laboratories should look upon this as a most important and essential part of his duties and this conception should be the mainspring of his relation to the laboratory resident. He should be ever ready to encourage such inquiries and to assist in their elucidation; and the whole purpose and tenor of the laboratory service should be to focus attention and maintain the interest of the future clinician—not upon tests and their technic, but upon their clinical significance and interpretation.

There are few residents, indeed, who will be unresponsive; few who will not feel that the laboratory service is of concrete clinical value, and none—it is to be hoped—who will fail to benefit in some measure and to apply the benefit to their future patients.

Occasionally, among them will be found the germ of a future clinical pathologist to carry on through others yet to come the enthusiasm impaired to himself. —*R. A. K.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

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*Chronic Intestinal Stasis**

AMERICAN writers have at no time been as thoroughly convinced of the etiologic relationship of intestinal stasis to a large proportion of those ills to which flesh is heir, as have many English writers who have become fired with the enthusiasm of those pioneers in stasis, Sir Arbuthnot Lane and Sir Arthur Keith. Some wag has said that this is because Americans have more room in which to exercise and thus prevent stasis than have our less fortunate cousins on the "straight little isle."

Intestinal stasis and intestinal toxemia undoubtedly do exist but we have come to intentionally avoid both terminologies in our diagnostic discussions. Intestinal toxemia is so easy an explanation of manifold ills and so thoroughly satisfies the patient that the use of this concept undoubtedly tends to dull one's diagnostic fervor and to promote its use when more thorough examination would have shown an entirely different cause for the complaint under study.

And thus we have perhaps gone rather to the other extreme in blocking out any consideration of stasis as an important factor in the causation of various diseases.

Jordan has attacked the problem from a radiodiagnostic viewpoint and has accumulated a wealth of interesting material. His work is abundantly and clearly illustrated, chiefly with x-ray pictures of the various conditions discussed.

While we feel that he has ascribed too many and too diverse ills primarily to intestinal stasis, we must confess that the proposition is put in a thoroughly concise and interesting way and that many of the general concepts are deserving of interest. In this country surely we have not as yet agreed that duodenal and gastric ulcer, carcinoma of the stomach, cardiospasm and carcinoma of the lower end of the esophagus are dependent ultimately upon chronic intestinal stasis nor does our understanding of carcinoma of the breast or of the upper esophagus as yet include an antecedent devitalization of the tissues through intestinal stasis.

However, as we have stated, we have perhaps gone too far in our efforts to avoid the pitfall of easy diagnosis and might well review the hypothesis championed by Jordan, provided we do not allow ourselves to be swayed too strongly thereby.

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*Chronic Intestinal Stasis. A Radiological Study. By Alfred C. Jordan, C.B.E., M.D., M. R. C. P. (Lond.) Cloth. Pp. 230. Price $7.50. Henry Frowde and Hodder and Stoughton, London, 1923.

*The Pathology and Treatment of Diabetes Mellitus**

TO those interested in diabetes the author requires no introduction. This book incorporated the Goulstonian lectures on glycemia and glycosuria and is written in the easy rhetoric of the lecture room rather than in accordance with the more formal textbook technic. The first third of the book is devoted to the physiology and pathology of the metabolism of sugar within the body, and no matter what the future developments in treatment consequent to the introduction of insulin may be, this portion of the book will possess an unaltered value.

The author has in the past applied undernutrition therapy, using his own dietary routine, and he emphasizes that with insulin, undernutrition must still be practiced, at least enough so that the fasting blood sugar shall reach normal limits each day. This is the only way in which we may hope for permanent improvement in the functional capacity of the pancreas. The author's treatment of coma is essentially the same as that applied in America. He has not stressed the importance of obesity as a predisposing factor in the causation of diabetes to such an extent as does Joslin.

An appendix contains sample dietaries, tables of food values, metabolism charts, description of laboratory methods, and a consideration of the ketogenic-antiketogenic ratio.

The volume, particularly that portion dealing with the physiology of carbohydrate metabolism, should be valuable to all who have any special interest in the study or treatment of diabetes.

Diabetes, Its Treatment by Insulin and Diet. A Handbook for the Patient†

WHEN we consider the relatively large number of brochures on diabetes for the use of physician and patient alike, published within the last few years, especially since the advent of insulin, we cannot but remark on the individualization which is apparent in the works by the various authors. Of those which the reviewer has seen so far, all are sufficiently different to be of individual interest, and one who is particularly interested in the treatment of diabetes will do well to have an assortment of these small books at his disposal. Each author presents his own method of attacking the problem.

In Petty's handbook we may mention the following outstanding features which we find discussed perhaps better here than in books by other authors.

The method of estimating the optimal diabetic diet is described with unusual clarity and is considerably simplified. While of interest to the patient, this should be read particularly by the physician.

In addition to the short chapter on insulin we find a description of the technic of administering insulin at home, care of the syringe and needle,

*Pathology and Treatment of Diabetes Mellitus. By George Graham, M.A., M.D., F.R.C.P. Cloth. Pp. 188. Price $2.00. Henry Frowde and Hodder and Stoughton, London, 1923.

†Diabetes, Its Treatment by Insulin and Diet: A Handbook for the Patient. By Orlando H. Petty, B.S., A.M., M.D., F.A.C.P Illustrated. Pp. 111. Cloth. Price $1..50. F. A. Davis Co., Philadelphia, 1924.

measurement in the syringe, etc. The simple menus and the daily diet schedule follow quite closely those originally drawn by Joslin.

We are glad to note here for the first time that the so-called 5 and 10 per cent vegetables are denominated 3 per cent and 6 per cent, which is their actual percentage when prepared for the table. Five and 10 per cent fruits are, of course, still designated as such.

There are several unusual and useful food tables, such as one showing foods of high fuel value, medium fuel value and low fuel value; one on the salt content of foods, and one showing excess of acid-forming or base-forming elements in foods.

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*Hemorrhoids**

TWO major motives have impelled the author to write a small monograph on hemorrhoids; first, the recognized confusion not only in the mind of the laity but also in a large proportion of physicians as to the true significance of the term "piles" (various diseases of the rectum and anus being grouped under this single heading); and second, as a vehicle for the description of his technic in the injection treatment of internal hemorrhoids. His treatment is distinctly conservative in that it avoids the necessity for extensive operation and usually saves the patient the expense and the time wasted in the hospital. The sections on surgical anatomy and physical diagnosis are well illustrated and a study thereof will serve to clear up misconceptions among those practitioners who are not strictly proctologists.

The advantages of the treatment of hemorrhoids by injection are not only the considerable saving of time and expense to the patient, but also the avoidance of general anesthesia, scarcely even a local anesthetic being required; the fact that symptoms cease immediately; that it is a safe procedure in pregnant women or individuals with advanced cardiac or renal disease; that there is as a rule no after-pain; that complications are less common; and that by this method hospital beds are left free for more urgent cases.

Pathological Technique†

THE histopathologists and bacteriologists *vade mecum* of laboratory methods, to which are added chapters on examination of the blood, urologic technique, examination of the cerebrospinal fluid, of sputum and a description of the technic of postmortem examination. This is a volume which, being brought up-to-date with each new edition, has survived for twenty-seven years the date of its first publication. It needs no introduction to the profession.

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*Hemorrhoids. By Arthur S. Morley, F.R.C.S., Eng. Cloth. Pp. 114. Henry Frowde and Hodder and Stoughton, London, 1923.

†Pathological Technique: A Practical Manual for Workers in Pathological Histology and Bacteriology. By Frank B. Mallory, M.D., Pathologist to the Boston City Hospital and James B. Wright, M.D., Pathologist to the Mass. General Hospital. Eighth edition, revised and enlarged. Octavo of 666 pp., with 180 illustrations. Cloth. Price $6.50. W. B. Saunders Co., 1924.

The American Society of Clinical Pathologists

Dr. John A. Kolmer, Philadelphia, Pa.
President

Dr. Frederick E. Sondern, New York
1st Vice President

Dr. W. F. Thomson, Beaumont, Texas
2nd Vice President

Dr. Ward Burdick, Denver, Colorado
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The Next Annual Meeting Will Be Held in Philadelphia May 20-23, 1925
Prepare for the Coming Convention

A letter and questionnaire have been sent out to all our members apprising them of our next annual meeting which is to be held May 20, 21, 22 and 23, 1925, at the Benjamin Franklin Hotel, in Philadelphia, the home city of our President, Dr. Kolmer. Under his inspiration the gathering this year bids fair to excel our previous successful conventions in scientific contributions to our specialty and advancement of our cause. A new and useful feature will be the commercial exhibit of instruments, apparatus and reagents for the laboratory worker. Members should make plans now for attending our next meeting. Dr. Burdick will be pleased to make hotel reservations not only for the Philadelphia meeting of the A. S. C. P. but he is also in a position to extend the service to include reservations in Atlantic City for the A. M. A. convention which is held the week following ours. Those who have papers to present, and we hope there will be many, will communicate at once with the secretary, Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado.

Members of the A. S. C. P. are requested to notify the secretary of any changes in their addresses.

Clinical pathologists desiring to apply for membership may communicate with the secretary, Dr. Ward Burdick, Children's Hospital, Denver, Colorado.

STANDARDS FOR CLINICAL PATHOLOGIST

One of the questions to be taken up at the business meeting at the next annual convention of the American Society of Clinical Pathologists is that relating to the qualification of clinical pathologists to practice this specialty. Considerable attention has been given by the society to this subject. In order to gather the consensus of opinion, the following letter and enclosures have been sent to every member.

Dear Doctor:

The incompetent and irresponsible laboratory is one of the evils now besetting the Medical Profession. The American Society of Clinical Pathologists has assumed the task of eradicating this excrescence on our professional body by endeavoring to institute standards of qualifications for those assuming to make laboratory diagnosis.

A preliminary report on laboratory standardization was submitted at the Rochester meeting by Dr. F. E. Sondern, Chairman of the Committee on Standardization (published in the January, 1925 number of the Journal of Laboratory and Clinical Medicine). Since then the committee has spent much time and labor in evolving a tentative plan for consideration at our next meeting. In order that every member may have an opportunity to study the details of the proposed plan, a copy is herewith enclosed. Appended thereto is a questionnaire which I would urge you to use for a free expression of your views and counsel, and as a guidance for the formation of a more permanent scheme. Let me hear from you at your early convenience.

I might add that the Philadelphia meeting promises to be a pronounced success. From present indications we shall have a wealth of interesting papers. If you have not already sent in the title for a paper you desire to present, do it now. One day of the convention will be devoted entirely to questions affecting the status of our members and our specialty.

Hoping to hear from you soon, I am

Fraternally yours,
(Signed) Ward Burdick,
Sec'y.-Treas.

A SYSTEM OF LABORATORY APPROVAL PROPOSED TO THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

By the Committee on Standardization

At the last meeting of the Society it was proposed that your Committee on Standardization frame the conditions under which the Society may issue Certificates of Approval to the members requesting the same.

The arguments in favor of the establishment of a method of approval of laboratories by a representative body such as the American Society of Clinical Pathologists are detailed in the report of your Committee adopted at the last meeting in Rochester, Minnesota, in June, 1924, which was essentially as follows:

It is an established fact, that a large number of clinical laboratories are conducted by persons absolutely unqualified by training and experience.

This state of affairs has, during recent years, engaged increasing attention particularly of those interested in public health, and a remedy is eagerly sought.

Practically speaking, clinical laboratory work is the result of a demand of the physician for aid in diagnosis, and were physicians as a class exacting in the laboratory service rendered, the existing evil would soon come to an end.

These constantly increasing justified complaints relative to inefficient and misleading laboratory service particularly in our larger cities must have the serious attention of such

bodies as our Association, and a remedy should be found to make every probable legislative control unnecessary or to guide such control if in public opinion it should become necessary.

Specific happenings in this connection may tend to emphasize the brief statements which have been made. Some eight years ago or more the New York City Commissioner of Health, Haven Emerson, called attention to the large number of complaints of incompetent work done by many private laboratories in New York City and expressed the hope that the New. York Academy of Medicine might do something to remedy this condition. In consequence, the Public Health Committee of the Academy made an investigation and as a result of this, advised that the Board of Health frame rules and regulations under which private laboratories should be allowed to operate. Beyond the requirement of a license, nothing has been done to date.

About the same time a similar complaint was made by officers of the American Medical Association to some of the private clinical pathologists of Chicago, with an unofficial request that the laboratory men themselves establish an organization for laboratory standardization and control, which, if suitable and efficient, should win the support of the American Medical Association. A determined effort in this direction was made under the leadership of Hektoen and others, but the World War prevented the success of this undertaking. It was also about this time that a study of this problem was made by the New York Bureau of Municipal Research, and in an exhaustive report published by this body the authority of the City Department of Health in the matter was demonstrated under existing law and compulsory control was strongly advocated in the interest of public health. The hospital surveys under the auspices of the American College of Surgeons, and the activities of the Council of Medical Education and Hospitals of the American Medical Association, have given increasing attention to laboratory personnel and equipment.

Enough has been said to prove that the danger of existing laboratory conditions is generally recognized and therefore, it scarcely seems necessary to review in detail evidence of its actual existence. There is no one active in clinical pathology who cannot detail a number of laboratories conducted by insufficiently trained technicians, and even by medical graduates who have scant knowledge of laboratory technic and employ incompetent technicians to do the actual laboratory work. It is also not uncommon to find hospital laboratories equally inefficient for like reasons, to say nothing of instances when a competent clinical pathologist, through stress of private work, exerts no control over his inefficient institution laboratory.

Admitting existing conditions, what can our Society do to remedy these evils, and what will happen if the issue is evaded? These are important questions with professional and economic significance which confront the qualified physician who specializes in clinical pathology, and it would seem urgent for our Society to assume as one of its legitimate functions, the duty of at least attempting a solution. While self-appointed censorship is on the whole an undesirable method, it does not stand without precedent, and if no other scheme is available, it may be necessary to come to it.

It is suggested that a method shall be devised by which the laboratories of members of the Society may, on request, secure what may be known as a "Certificate of Approval" of the American Society of Clinical Pathologists. This method should include qualifications of personnel, standardization of laboratory procedure controlled by inspection, periodic report on submitted specimens or by such other method as the Society may deem proper. The details of these requirements and standards might be relatively simple at first, to be improved and perfected as the plan develops and its need and desirability become more evident. At all events, this official approval should be of such high standard as to secure at sight the unqualified endorsement of such bodies as the American Medical Association, the American College of Surgeons, etc.

This procedure, to be sure, will not eliminate the inefficient laboratory because it will not succeed in making the careless or incompetent physician or the lay public pay proper attention to the securing of efficient laboratory service. It will however, convince the thinking medical men of the country and the leading organizations of which they are members, that a laboratory standard has been established which they cannot ignore.

We live in an era of profuse legislative activity in the control of almost every human endeavor, and if this should extend to a consideration of laboratories, aside from such activity as a purely medical professional function, as it well may, the existence of an established standard endorsed by leading medical organizations might easily shape a proper course of action. In the absence of such organization, however, the legislation might easily grow to bizarre proportions, of which we have so many examples in other fields at the present time.

The only existing practical standardization and control of public health laboratories is that of the New York State Department of Health. This plan was conceived and elaborated by Dr. A. B. Wadsworth, Director of the Laboratories of that Department and carried out by and under the supervision of Dr. Ruth Gilbert, head of the Diagnostic Laboratory of the same. The system is of decided value to the Department in estimation of the reliability of the laboratories throughout the State and their value in safeguarding public health. It is in the main characterized by courteous cooperation and constructive criticism in which way it renders a valuable service to the laboratory itself; and its existence without any doubt urges higher laboratory standards.

A similar system of voluntary submission to laboratory control exerted by this Society may easily be the means of convincing the thinking members of the medical profession that an absolute laboratory standard has been established.

It will be of little or no use unless a large proportion of the efficient laboratories of the country join in the movement and they will not do so unless the control is exercised by competent authority in a tactful, courteous and constructive manner. In addition it must also be recognized by the large influential medical societies, and their emphatic publicity is necessary to make those disinclined submit to approval in the interest of the common good.

Your committee recommends the following:

CONDITIONS UNDER WHICH THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS WILL ISSUE CERTIFICATES OF APPROVAL TO THE LABORATORIES OF MEMBERS REQUESTING THE SAME.

Certificates shall be issued for the term of one year, unless revoked, for those branches of clinical pathology for which the applicant has applied and in which the laboratory is considered proficient. Certificates to be in form as per Exhibit I.

Proficiency is to be determined by the Society as its appointed representatives elect. Full cooperation is expected of the applicant, and courteous, just and constructive criticism is to be the rule of the Society.

As a basis for approval, the applicant is to submit the details requested in the application as per Exhibit II. To furnish quarterly reports on the work done by the laboratory during the three months just passed, on suitable blanks as per Exhibit III. To subscribe to certain rules and standard methods and to furnish details of technic employed as per Exhibits IV.* (It will be noted that for the present the latter apply only to examinations which have to do with communicable disease and the examinations of milk and water as these are considered most important. It is expected that similar details and agreements will be arranged later to cover all the branches of clinical laboratory work.

The laboratory shall be subject to inspection by competent authority to verify the various agreements to which the Director has subscribed.

From time to time test specimens will be sent to the laboratory for examination and report which are to have the same attention in examination and report as specimens submitted to the laboratory in the regular course of its work.

Applicants for approval are also expected to subscribe to the following rules:

The Director of the clinical laboratory must be a licensed physician and all diagnostic opinions of the laboratory must be framed by him or with his ap-

*Exhibit form IV eliminated from this preliminary survey.

proval. In his absence this shall be done by an equally qualified associate or substitute. The Director shall have complied with all federal, state, county and city regulations which apply to laboratories. It shall be considered unethical to advertise the laboratory in any way other than that having the approval of the Society. Directors, consultants and the like shall not be announced unless they are actually on duty in the laboratory. The division of fees with physicians and others who secure work for the laboratory is prohibited. The equipment of the laboratory shall be such that the proper technical procedures can be carried out in every instance and includes ready access to the necessary scientific books and periodicals.

A fee will be expected from each laboratory requesting approval to cover the acutal expense to the Society, this to be as reasonable as efficient work allows.

The success of this plan depends on the complete support of the influential laboratory men of the country. With such cooperation the Society can appeal to the American Medical Association, the American College of Surgeons and similar bodies for several purposes, viz.:

To endorse this effort to secure known reliable laboratory service for physician and patient; to publish lists of the approved laboratories, and chiefly to educate the physician to the need of approved laboratories, and to point out the danger of accepting the work of individuals not approved.

In event of proposed legislation for the control of laboratories if necessary at all, this plan, seasoned by then, can be offered as a compulsory instead of a voluntary method.

It is not hoped that this method of approval and control will eliminate the unfit worker or laboratory, but if the plan is given sufficient publicity those not approved will have difficulty in defending their position.

COMMITTEE ON STANDARDIZATION

FREDERICK E. SONDERN, M.D., Chairman, *New York City, New York.*
A. V. ST. GEORGE, M.D., *New York City, New York.*
W. F. THOMSON, M.D., *Beaumont, Texas.*
GEORGE IVES, M.D., *St. Louis, Missouri.*
THADDEUS WALKER, M.D., *Boston, Massachusetts.*
RUTH GILBERT, M.D., *Albany, New York.*
HARRY J. CORPER, M.D., *Denver, Colorado.*

QUESTIONNAIRE

1. Do you approve of the plan outlined by the Committee on Laboratory Standardization?
2. If not, state objections.
3. What modifications or additions would you suggest?
4. Remarks:
5. Further Comments—(write freely).

NAME .....

ADDRESS .....

.....

Some very interesting replies have been received in response to the questionnaire which will be discussed in a future issue.

APPLICATION FOR CERTIFICATE OF APPROVAL

Date .....

To the Committee on Standardization of the American Society of Clinical Pathologists:

I, ... , M.D.,

located at .....
P. O. Address City State

hereby apply for a certificate of approval of my qualifications in Clinical Pathology on the grounds of the following education and experience:

SCIENTIFIC AND MEDICAL EDUCATION

Name of School	Location	Time		Degree
		From	To	

SPECIAL COURSES

Institution	Location	Time		
		From	To	

PRACTICAL EXPERIENCE

Laboratory	Character of Work Done	Time	
		From	To

The laboratory staff consists of .....

.....

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(EXHIBIT II.)
(Obverse side)

(over)

The laboratory has the following equipment in good condition:

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The scope of work of the laboratory includes .....

.....

.....

.....

.....

Indicate by means of a cross (x) the classes of service you are performing.

Private... Hospital... Medical School... Municipal ... County... State... Federal...

I hereby agree that:

1. The work will be done strictly in accordance with the code of ethics of the American Society of Clinical Pathologists.
2. The technic employed will be that approved by the Society.
3. An accurate record will be kept of the daily work.
4. All stained tissue sections will be kept on file.
5. The laboratory will be open for inspection to representatives of the Society.
6. Specimens sent from the Society for testing the efficiency of approved laboratories will be examined and reported.

.....

Signature of Applicant.

(REVERSE SIDE)

CERTIFICATE OF APPROVAL

Issued by

The American Society of Clinical Pathologists

This is to certify, That

_____ **M. D.**

has demonstrated to the Committee on Standardization of The American Society of Clinical Pathologists that he is competent to perform the necessary examinations and interpret the findings in the following branches of Clinical Pathology:

Clinical Microscopy,

Clinical Chemistry,

Bacteriology,

Serology,

Tissue Diagnosis.

COMMITTEE ON LABORATORY STANDARDIZATION

_____

_____

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_____

[SEAL]

Dated _____

_____
President.

_____
Secretary.

(EXHIBIT I)

A Four Day Convention

Owing to the large number of excellent scientific papers that will be presented at the next convention and in order to allow more time for the discussion of business matters, the Executive Committee of the American Society of Clinical Pathologists has extended the convention one day ahead, beginning Wednesday, May 20th, and lasting through Saturday, May 23, 1925. All reservations at the Benjamin Franklin Hotel have been automatically changed to include the new date.

Program of the Fourth Annual Meeting of the American Society of Clinical Pathologists

Benjamin Franklin Hotel, 9th and Chestnut Streets, Philadelphia, Pennsylvania.

WEDNESDAY, MAY 20, 9 A. M. (PROMPTLY)

Call to Order—Short Business Session

Scientific Program

The Preparation of Solutions of Dextrose for Intravenous Administration, by Dr. Ralph G. Stillman, New York City, N. Y.

Utilization of Carbohydrates, by Dr. W. G. Karr, Philadelphia, Pennsylvania (by invitation).

Diagnostic Value of Spinal Fluid Sugar Content, by Dr. W. Parker Stowe, Rochester, New York.

Alveolar CO_2 Tension and Acetone in the Expired Air in Acidosis, by Dr. Paul Roth, Battle Creek, Michigan.

WEDNESDAY, MAY 20, 2 P. M. (PROMPTLY)

Aids to Urinalysis in a Large Hospital, by Dr. C. Pons and Dr. E. B. Krumbhaar, Philadelphia, Pennsylvania (by invitation).

A New System of Duplicating and Placing Laboratory Reports in the History, by Dr. Frank W. Hartman, Detroit, Michigan.

Value of Method of Keeping Records of Tissue Examinations, by Dr. L. A. Turley, Norman, Oklahoma.

A New Type of Instrument for the Estimation of Hemoglobin, by Dr. C. E. Roderick, Battle Creek, Michigan.

Some Useful Apparatus, by Dr. Max Shaweker, Dover, Ohio.

The Organization of a Metabolism Laboratory, by Dr. William H. Stoner, Philadelphia, Pennsylvania.

Endothelioma; the Use and Misuse of the Term, by Dr. James B. Bullitt, Chapel Hill, North Carolina.

Myocardial and Endocardial Changes in Cardiac Defects, by Dr. Maude E. Abbott, Philadelphia, Pennsylvania.

THURSDAY, MAY 21, 9 A. M. (PROMPTLY)

Autopsy Report of Two Cases of Thymic Death During Surgical Operations, by Dr. Jessie W. Fisher, Middletown, Connecticut.

Some Enzyme Studies with Desiccated Gonadal Tissue, by Dr. Herman Sharlit and Dr. William G. Lyle, New York City, N. Y.

A Comparison of Enzyme Actions of Tumors and Normal Tissues, by Dr. K. George Falk and Helen Miller Noyes, New York City, N. Y., (by invitation).

A Double Hydrogen Electrode System for the Determination of Hydrogen-Ion Concentration, by Dr. George H. Meeker and Mr. Bernard Oser, Philadelphia, Pennsylvania, (by invitation).

Observations on the Dick Test, Toxin Immunization and Serum Treatment of Scarlet Fever, by Dr. John A. Murphy, Philadelphia, Pennsylvania. (by invitation).

Pneumococcus Antibody Solution, by Dr. Frank M. Huntoon, Glenolden, Pennsylvania.
Variation in Size of Red Blood Corpuscles and High Color Index with little or no Anemia, (Lantern Slides), by Dr. Mortimer Warren, Portland, Maine.

THURSDAY, MAY 21, 2 P. M. (PROMPTLY)

Normal and Pathologic Basal Metabolic Rate in Obesity, by Dr. Horry M. Jones, Chicago, Illinois.
Studies on Blood Cultures with Special Reference to the "Massive" Method, by Dr. Herbert Fox and Dr. William G. Leaman, Philadelphia, Pennsylvania.
A Comparative Study of Liver Functional Tests, by Dr. A. I. Rubenstone and Dr. Louis Tuft, Philadelphia, Pennsylvania.
The Bactericidal Action of Whole Blood as Determined by the Heist-Lacy Method, by Dr. B. S. Parks, Philadelphia, Pennsylvania, (by invitation).
Studies on the Bacteriology of the Urine in Cooperation with Catheterization of the Ureters, by Dr. Robert A. Keilty, Danville, Pennsylvania.
Study of Cases of Acute Leukemia and Acute Mononucleosis, by Dr. B. L. Crawford, and Dr. Harold W. Jones, Philadelphia, Pennsylvania.
Blood Counts in Mississippi, by Dr. Leon S. Lippencott, Vicksburg, Mississippi.

THURSDAY, MAY 21, 7 P. M. (PROMPTLY).

Reaction After Typhoid Vaccination, by Dr. Henry J. Nichols, Washington, D. C.
The Measurement of Cloudiness in Liquids, by Dr. William G. Exton, Newark, New Jersey.
Studies in Embalming Fluids in Relation to Gross and Histological Tissue Examinations, by Dr. John A. Kolmer and Dr. Fred Boerner, Philadelphia, Pennsylvania.
The Specific Inflammatory Reaction of Immunized Animals (Arthus Phenomenon), by Dr. Eugene L. Opie, Philadelphia, Pennsylvania, (by invitation).
Methods of Staining Tubercle Bacilli, by Dr. H. J. Corper, Denver, Colorado.

FRIDAY, MAY 22, AT 9 A. M. (PROMPTLY)

Value of the Leucocyte Fragility Test in the Prognosis of Pneumonia, by Dr. C. Pons and Dr. E. P. Ward, Philadelphia, Pennsylvania, (by invitation).
Sedimentation Rate of Erythrocytes, by Dr. H. N. Cooper, Watertown, New York.
Standardization of Tuberculin, by Dr. Joseph D. Aronson, Philadelphia, Pennsylvania, (by invitation).
Bronchial Spirochetosis, by Dr. Thomas L. Ramsey, Toledo, Ohio.
Etiological Studies in Psoriasis, by Miss Mary Marcus, Philadelphia, Pennsylvania, (by invitation).
Squamous Cell Carcinoma of the Gall Bladder, by Dr. Frank W. Hartman, Dr. W. E. Johnson, Detroit, Michigan.
The Technic of the Practical Application of the Pathogen Selective Cultural Method, by Dr. A. I. Rubenstone, Philadelphia, Pennsylvania.

FRIDAY, MAY 22, 2 P. M. (PROMPTLY).

A System of Laboratory Approval Proposed to the American Society of Clinical Pathologists, by Dr. Frederick E. Sondern for the Committee on Standardization.
Discussion to be opened by Dr. Ruth Gilbert, Dr. C. Y. White (by invitation) and Dr. S. R. Haythorn.

Open Discussion

How Can We Best Promote the Objects Contained in Article II of Our Constitution? By Dr. Herman Spitz, Nashville, Tennessee.
Suggested Method to be Followed in Developing a Standardized Course for Medical Technicians, by Dr. Walter E. King, Detroit, Michigan.

FRIDAY, MAY 22, 7 P. M. (PROMPTLY)

Annual Dinner in Ball Room of the Benjamin Franklin Hotel.

Presidential Address

The Functions of a Hospital, by Dr. George H. Meeker, Dean of the Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

SATURDAY, MAY 23, 9 A. M. (PROMPTLY)

Business Session

SATURDAY, MAY 23, 2 P. M. (PROMPTLY)

The Clinical Significance of Anticomplementary Serum, and Spinal Fluids in the Wassermann Reaction, (Kolmer Modification), by Dr. A. H. Sanford, Rochester, Minnesota.

A Standardized Wassermann Report, by Dr. A. J. Casselman, Camden, New Jersey.

The Present Status of the Kolmer Complement-Fixation Test for Syphilis as Determined by a Comparison with Numerous Other Methods, by Dr. Robert A. Kilduffe, Atlantic City New Jersey.

Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test, by Dr. Robert G. Owen, Detroit, Michigan.

Kahn's Precipitation Reaction as Compared to Kolmer's Complement-Fixation Test, by Dr. A. S. Giordano, South Bend, Indiana.

A Clinical Study of the Kolmer and Kahn Reactions in Syphilis, by Dr. R. L. Kelly, Louisville, Kentucky, (by invitation).

The Meinecke Reaction as Compared with the Wassermann in One Thousand Specimens of Blood Sera. (Lantern Slides), by Dr. A. M. P. Saunders, Dunning, Illinois.

Scientific Exhibit

Microscopic and Other Specimens Relating to Tropical Medical Problems, by Dr. J. M. Feder, Santo Tomas Hospital, Panama.